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NIEHS Science Open House Poster Sessions: Abstracts

Methods for Detecting Mutagenic Exposures and Genetic Damage in Man.

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Traditional epidemiologic studies for genetic toxicity have been generally limited to reliance on measurement of spontaneous abortion, congenital defects, or malignancy as indicators of genetic damage. These measures, however, are not specifically related to genetic damage, may occur only after long latency or are likely to be observed rarely and thus require very large populations for effective study. In addition, there is frequently considerable uncertainty about the identity, number, and dose of compounds to which humans are exposed.

Toxicologic studies have the power of the experimental method, but they can be difficult to interpret in terms of relevance to human exposure and potential for human damage. Reasons include species specificity, uncertainty in extrapolating dose-response relationships, and difficulty in dealing realistically with effects of multiple exposures or competing risks as they might apply to man.

To complement traditional epidemiologic and toxicologic methods, we are developing laboratory assays to help define human exposures, susceptibilities, and outcomes, as well as the mechanisms by which they are related.

This presentation catalogs potential clinical studies and laboratory tests for detecting mutagenic exposures and genetic damage in man. The catalog includes rough estimates of likely population sizes required to use these assays, latency between exposure and the first observable effects, as well as the period it is likely that the effects can continue to be identified, feasibility, and cost.

In addition, results from three studies involving the development or application of these techniques are presented. One of these studies, involving use of an autoradiographic assay, showed that substantially more peripheral blood mononuclear cells from patients receiving chemotherapy for breast cancer than from controls were resistant to 6-thioguanine (6-TG) after short-term culture. These resistant cells, however, could be accounted for by cells not requiring stimulation by phytohaemagglutin. The high proportion cells resistant to 6-TG that do not require phytohemagglutin (about 5%) suggests that quantitating resistant cells does not specifically estimate genetic damage. A second study confirmed the previously reported presence of mutagenic substances in urine of smokers, but found no evidence to support a recent study suggesting the presence of mutagenic substance in urine of patients with cirrhosis. We suspect that residual histidine (causing growth of the background lawn and increased number of spontaneous revertants) rather than mutagenic materials may have been responsible for results initially observed among specimens from cirrhotics, and suggested techniques that could exclude this possibility in future studies of urine mutagenicity. The third study sought evidence of mutagenic material in urine of subjects with bilharzial associated bladder cancer in Egypt. The presence of mutagenic substances in these assays could only be excluded by devising tests for quantitating bacterial growth in the background lawn of plates used in the Ames assay. Taken together, these three studies demonstrate that interpreting laboratory tests for mutagenic exposures or genetic damage in man requires understanding and careful investigation of methodological and technological issues affecting these assays.

Implication of Nonlinear Kinetics on Risk Estimation in Carcinogenesis.

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For chemicals whose metabolites interact with DNA, it may be biologically more meaningful to relate tumor response to the cellular levels of these adducts rather than to applied dose. Substantial experimental data suggests that this relationship is linear. An implication of such a linear relationship is that the nonlinearities of the dose-response curve for tumor induction are due to the kinetic processes involved in the formation of carcinogen metabolite–DNA adducts. Of particular importance is the possibility that the kinetic processes may exhibit nonlinear "threshold"-like behavior which results from saturation of detoxification or DNA repair processes.

NIEHS Data Communication.

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During the past five years, data communication at NIEHS has grown in primarily a linear fashion. For example: the number of modems installed on local computers has increased from zero to more than 48; the number of local terminals has increased from three to more than 75; the number of local and remote computers has increased from three to more than ten; and terminal access has changed from acoustic couplers at 30 characters per second to dial modems at 120 characters per second. Recent analysis has shown that more linear growth will not allow NIEHS to overcome the limitations of its

current data communication methods. More sophisticated and complex methods using more modern technologies will be required.

Analysis has also shown that the desired improvements can be divided into six distinct projects, providing improved remote job entry capability from the NIEHS computer to various remote mainframe computers, access to local computers with local terminals, access to remote computers with local terminals, access to local computers with remote terminals, a data communication network of local laboratory minicomputers and a data communication network of remote laboratory minicomputers.

The six projects will be accomplished with a variety of new technologies in mind including a digital private branch exchange, an intelligent port selector, portocol converters, concentrators, limited distance modems, direct leased lines, and auto dialers. The implicatios of these new technologies on three of the six projects are discussed.

The Early Pregnancy Study. ALLEN J. WILCOX, *Epidemiology Branch, Biometry and Risk Assessment Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The purpose of this study is to develop a method for measuring early human pregnancy loss, in order to detect damage that might be inflicted on the early fetus by environmental toxins. Very early pregnancy loss can end in a flow similar to menses and thus be undetected. There is evidence to suggest that a third of all pregnancies end in this way. The extent to which this early loss may be related to environmental insult is unknown. Observation of experimental animals has shown that early reproductive processes are sensitive to environmental toxins, but it has not been possible to observe early human pregnancy so directly.

This study will measure the monthly occurrence of conception and subsequent early fetal loss by analyzing daily urine specimens collected by women who are planning to become pregnant. Trophoblastic tissue of the conceptus begins to produce human chorionic gonadotropin (hCG) about 7 to 10 days after conception. Recent laboratory developments have led to a hCG radioimmunoassay that is far more sensitive and specific than any previously available. By using this new assay, we can document the risk of early fetal loss with considerable accuracy. In the pilot phase of this study, daily urines will be collected from about 30 women until the women are clinically pregnant. A subsequent study will enroll several hundred women, among whom common exposures such as smoking and alcohol consumption can be examined for possible effects on early pregnancy. Eventually, it may be possible to extend this method to high-risk groups of women in occupational or other settings where toxic effects on early pregnancy are suspected.

Patterns of Tumor Incidence and False-Positive Rates in Two-Year Carcinogenesis Bioassays. JOSEPH K. HASEMAN, *National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Patterns of tumor incidence in 25 of the National Toxicology Program's (NTP) two-year cancer bioassay feeding studies in Fischer 344 rats were investigated. It was found that the overall frequencies of statistically significant ($p < 0.01$) increases and decreases in organ-specific tumor incidence in treated groups relative to controls were approximately the same. The decreases were due primarily to mammary gland fibroadenomas in females (which were clearly associated with decreased weight gain in the treated groups) and leukemia/lymphoma in both sexes (which were frequently associated with increased liver tumor incidences in the treated groups). A clear explanation for this latter association was not apparent.

False-positive rates in the NTP two-year carcinogenesis bioassay were also examined. Previous studies that have reported unacceptably high (20-50%) false-positive rates for these bioassays were reviewed. It was shown that the decision rules used in many of these earlier investigations were far different than the procedures actually employed by the NTP in the interpretation of bioassay data. Data from recent NTP feeding studies were examined, and the statistical significance of observed tumor increases were compared with the final interpretations regarding the carcinogenic effect of the chemicals under study. Based on this examination, a more realistic decision procedure was formulated. By using this procedure and updated historical control tumor rates, it was shown that the actual overall false-positive rate in NCI/NTP bioassays appears to be no greater than 7–8%.

Analysis of Variability in the International Collaborative Study on Genetic Drift in Ames Tester Strains. BARRY H. MARGOLIN and KENNETH J. RISKO, *Biometry and Risk Assessment Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709,* and MICHAEL D. SHELBY and ERROL ZEIGER, *Toxicology Research and Testing Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Data from 38 laboratories using five Salmonella strains (TA98, TA100, TA1535, TA1537, and TA1538) to determine the mutagenic effects of 4-nitroquinoline-*N*-oxide were analyzed to answer a number of questions regarding test data variability. Each laboratory conducted tests according to a common protocol, using both its in-house cultures and a set of reference cultures provided to all laboratories. It was found that plate-to-plate and day-to-day variability within a laboratory did not differ substantially between the in-house and reference cultures for any strain; this indicated no difference in the laboratories' handling of the two cultures. As expected, plate-to-plate variability was substantially less than day-to-day variability, which, in turn, was substantially less than inter-laboratory variability. The solvent DMSO was found to have a small (6-7%) depressive effect on the spontaneous mutant frequency for the two plasmid-containing strains, TA98 and TA100, but not for the other three. When the mean value and variance of all laboratories for the in-house culture at each test dose and strain were compared with the corresponding reference culture values, no major differences were seen. Any increase in mean or variance in the distribution of laboratory means in one of the two cultures could be ascribed largely to a small number of laboratories. As a rule, laboratories that reported "high" or "low" levels of spontaneous or induced revertants per plate tended to deviate in the same direction for most strains and for both in-house and reference cultures. If "genetic drift" contributed to the interlaboratory variability in this collaborative study, it was a minor component.

The Breast Milk and Formula Project. W. J. ROGAN and B. C. GLADEN, *Biometry and Risk Assessment Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Persistent fat-soluble pesticides and industrial chemicals that have become widespread environmental pollutants appear with high prevalence in human milk. The best known of these are the pesticide DDT and the industrial insulators, the polychlorinated biphenyls (PCBs). In 1978, we began a prospective birth cohort study to see if there was illness in children attributable to the presence of these chemicals in human milk. About 850 children from three North Carolina institutions are being followed. We enroll families at or near term, examine the child and obtain a medical history at birth, and see the child periodically during the first years of life. In addition to history and physical, we do a standardized (Brazelton) behavioral assessment at birth, developmental (Bayley) scales at age two, and a "school readiness" (McCarthy) scale from age three on. We collect placenta, maternal and cord blood, and breast milk over the course of lactation. All biological and 10% of formula samples are analyzed for PCBs, DDE, and total organic chlorine and bromine. There are detectable amounts of PCBs and DDT (as DDE) in virtually all breast milk samples. Amounts decline over the course of lactation. Using amount of chemical in breast milk as an indicator of transplacental exposure, we see no evidence of toxicity at birth; however, cord blood values are lower than maternal blood, and thus little exposure may take place this way. Women with higher DDE levels (but not PCB levels) tend to breast feed for shorter lengths of time; this is not accompanied by any major difference in weight gain or illness frequency in the children and may be a maternal effect.

2,6-Xylidine-Induced Nasal Cancer in Rats. C. A. MONTGOMERY, MARY KORNREICH and JERRY HARDISTY, *Toxicology Research and Testing Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, 27709.*

2,6-Xylidine is a chemical intermediate used principally in the production of dyes. It is also a component of tobacco smoke, a degradation product of aniline based pesticides, and a metabolite of certain drugs, particularly the xylide group of local anesthetics. A carcinogenesis bioassay was conducted by the National Toxicology Program by administration of 2,6-xylidine in the diet to groups of 56 male and 56 female Charles River F_1 generation CD rats at dose levels of 0, 300, 1000 and 3000 ppm for 102 weeks. The F_0 parents of these animals received the test diet at the same doses before breeding, during pregnancy, and through the lactation period.

On chronic exposure, the epithelium of the nasal cavity was the primary target organ for both neoplastic and nonneoplastic lesions. The incidence of both carcinomas and papillary adenomas of the nasal cavity were statistically increased in high dose male and female rats when compared to controls. Carcinomas occurred in 50% of the high dose males, 43% of the high dose females and 2% of the medium dose females. Most of these malignant epithelial tumors appeared to arise from the submucosal glands in the dorsal posterior portion of the nasal turbinates. The carcinomas were highly invasive and frequently destroyed the nasal turbinate and nasal septum. Metastasis to the brain was present in 9% of the male and 13% of the female high dose rats. Papillary adenomas occurred in 18% of the high dose males, 4% of the medium dose males and 11% of the high dose females.

A striking result was the presence of malignant mesenchymal tumors of the nasal cavity. Two rhabdomyosarcomas occurred in high dose male rats and two in high dose female rats. Malignant mixed tumors with features of adenocarcinoma and rhabdomyosarcoma were present in one high dose male and one high dose female rat. One undifferentiated sarcoma was seen in a high dose female rat.

Nonneoplastic lesions observed in the nasal cavities of dosed rats included acute inflammation, epithelial hyperplasia, and squamous metaplasia. There was a dose-related increase in the incidence of acute rhinitis among control and treated groups.

The National Toxicology Program Pathology Working Group and Its Evaluation of Chemical Bioassays. C. MONTGOMERY and G. BOORMAN, *Toxicology Research and Testing Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The NTP pathology working group is a vital part of the pathology peer review system. It consists of five or six pathologists, one-half or more composed of NTP staff, and the remainder from industry, academia, private consultants or other branches of the government. These experts in rodent pathology review the quality and accuracy of pathologic data for each chemical. Emphasis is on use of proper neoplastic terminology, proper identification of the target organs of subchronic or chronic toxic exposure and clarification of the pathogenesis of toxic induced injury in the host.

Microslides are read in a blind fashion by the pathology working group without knowledge of the identity of the original pathologist, dose group or other data subject to bias. The quality of work performed by the original pathologist and quality assessment pathologist and the quality of histotechnique performed by the contract laboratory are all assessed by the work group, and specific recommendations are made.

In reviewing subchronic studies, the pathology work gorup makes an interpretation of each toxic lesion as to its life-threatening potential and by using these data recommends doses to be used in the chronic bioassay. Discrepancies in diagnosis are cited, and those slides are returned to the original pathologist for reconsideration and update of individual animal data records for compliance with the good laboratory practice act. The original pathologist has the ultimate responsibility for the study and therefore makes the final decision on micropathology diagnoses. However, if the working group disagrees with the final diagnosis, a separate set of data documenting this difference of opinion may be generated for inclusion in a NTP technical report. The NTP pathology working group is a highly respected, thorough, peer review system with one primary goal: that the pathology portion of NTP contracted chemical studies be accurate and scientifically sound.

Differential Estrogenic Response of the Pituitary and Uterus to Kepone (Chlordecone) in the Fischer-344 Rat. J. C. LAMB IV, M. D. ROSS, K. M. ALLEN, M. M. DOZIER and J. R. REEL, *Toxicology Research and Testing Program, National Toxicology Program, National Institute of*

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The insecticide, Kepone (K), has profound estrogenic effects on the reproductive system of birds and mammals. The observation that diethylstilbestrol (DES) implanted sc in Silastic tubing induced prolactin-secreting pituitary tumors and uterine growth in Fischer-344 rats suggested a useful animal model in which to further evaluate the estrogenic activity of Kepone. In the initial study, adult F-344 rats were ovariectomized and implanted SC with Silastic tubing containing sesame oil, 5 mg DES in oil, or 50, 100 or 200 mg Kepone in oil. Groups of rats were scheduled for bleeding and necropsy at 2, 4, 8, 12 and 16 weeks. In the second study, ovariectomized rats were implanted SC with tubing containing sesame oil, 0.5 or 5.0 mg DES in oil, or 50 or 100 mg Kepone in oil. Groups were scheduled for bleeding and necropsy at 2, 4 and 8 weeks. From 2 weeks to the conclusion of these studies there was a sustained evaluation of uterine peroxidase activity and a 3- to 6-fold increase in uterine weights in groups implanted with 0.5 or 5 mg DES or 100 mg Kepone, whereas animals that received 50 mg Kepone showed uterine responses about 50% that of the 100 mg Kepone group. Within 24 hr after SC implantation of 200 mg Kepone, all animals developed severe nervous tremors and died 3 to 4 days later. By 8 weeks many of the rats that received 5 mg DES developed pyometritis which was associated with a high incidence of mortality. In addition, liver weights increased progressively from 2 to 8 or 16 weeks in those animals treated with 5 mg DES or 100 mg Kepone. Further, 0.5 or 5.0 mg DES stimulated marked and progressive increases in pituitary weight and prolactin secretion, commencing at 2 weeks and persisting to the end of the studies. In contrast, neither 50 nor 100 mg Kepone increased pituitary weights; however, there was a transient elevation of plasma prolactin levels at 2 and 4 weeks. In conclusion, Kepone mimics at least two well known effects of DES on the uterus, but is largely devoid of the stimulatory effects of DES on the pituitary.

Proliferative Lesions of the Exocrine Pancreas in the Fischer 344/N Rat.

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There is concern that an increasing incidence of pancreatic cancer in man may be related to increasing exposure to environmental carcinogens. Experimental models of pancreatic carcinogenesis in rodents show that certain chemicals induce or enhance exocrine pancreatic cancer and that dietary factors modify the progression of pancreatic cancer. When a high incidence of proliferative lesions of the exocrine pancreas in male Fischer 344/N rats was observed in several recent carcinogenicity bioassays, a histopathologic review was initiated to develop diagnostic criteria and standardized nomenclature for those lesions. Since diets high in unsaturated fat enhance pancreatic carcinogenesis in experimental rodent models, pancreatic tissue from untreated control rats and control rats receiving corn oil were examined by light microscopy.

Lesions of the exocrine pancreas were classified as focal cellular change, focal acinar hyperplasia, acinar adenoma and acinar carcinoma. Foci of cellular change affect a small portion of the pancreatic lobule, are characterized frequently by cellular hypertrophy, enlarged nuclei, and altered staining properties, and do not compress adjacent parenchyma. Foci of hyperplasia compress adjacent tissue, show mild alteration of acinar architecture, and are continguous with unaffected acini. Acinar adenomas are discrete rounded nodules that usually are not contiguous with unaffected parenchyma. Carcinomas have typical changes indicating malignancy such as cellular anaplasia, altered growth pattern, invasion, and metastasis.

Preliminary results of our review indicate that proliferative lesions of the exocrine pancreas were more frequent in control rats receiving corn oil by gavage than in untreated control rats. There was a 10-fold greater incidence of focal acinar hyperplasia and almost a 3-fold greater incidence of adenoma in rats receiving corn oil compared to untreated controls. However, when the data are broken down by individual bioassays, the incidence of proliferative lesions was not consistent between groups of corn oil control rats. Although proliferative lesions of the exocrine pancreas apparently are associated at times with corn oil treatment, the variability between studies indicates that factors other than corn oil may be involved. Investigation of the cause(s) of these findings is currently in progress.

Purification and Quantitation by Radioimmunoassay of Two Forms of Cytochrome P-448.

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A second form of cytochrome P-448 has been isolated from livers of rats treated with 3,4,5,3′,4′,5′-hexachlorobiphenyl (3,4,5-HCB), a methylcholanthrene (3-MC) type inducer. The purified cytochrome (referred to as P-448_{HCB}) had a specific content of 18.8 nmole/mg and migrated as a single band on SDS-polyacrylamide gels. Two other forms of cytochrome P-450, P-450_{PB} and P-448_{MC}, were isolated from phenobarbital- and 3-MC-treated rats, respectively. Cytochrome P-448_{HCB} had a molecular weight of 52,000, similar to that of P-450_{PB}, but different from P-448_{MC} (55,000). The maxima of the coreduced difference spectra for cytochromes P-450_{PB}, P-448_{MC}, and P-448_{HCB} were 450, 447.5 and 448 nm, respectively. The three forms could also be distinguished by their catalytic activities, immunological properties, and peptide maps. An RIA was developed specifically for cytochromes P-448_{HCB} and P-448_{MC} for quantitation in the nanogram range in biological samples. This RIA indicated that both forms of cytochrome P-448 are induced markedly by 3-MC, 3,4,5-HCB and Aroclor 1254 in rat liver microsomes.

Changes in the Distribution and Excretion of Two Hexachlorobiphenyls in Senescent Rats.

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Polychlorinated biphenyls (PCBs) are industrial chemicals which have become widespread environmental pollutants. Their toxicity and persistence have made them a major health hazard, with PCB residues being detected in essentially all human populations. PCBs tend to accumulate in adipose tis-

sue with the rate of metabolism being the prime determinant of their persistence. Since both body composition and drug disposition have been reported to change with age, we investigated the distribution, metabolism, and excretion of two hexachlorobiphenyls (HCBs) in senescent rats. 2,3,6,2',3',6'-HCB (236) and 2,4,5,2',4',5'-HCB (245) are symmetrical isomers whose disposition in young rats has previously been studied in our laboratory. In the present study, senescent (23-24 month) male Sprague-Dawley rats were treated IV with ^{14}C-HCBs at 0.6 mg/kg, sacrificed from 1 hr to 21 days after treatment, and tissue distribution of ^{14}C-HCB-derived radioactivity determined. Urine and feces were collected daily and analyzed for radioactivity. The radioactivity in the major tissue depots—blood, liver, muscle, skin and adipose tissue—were extracted and chromatographed to determine parent HCB/metabolite ratios. 236 was distributed to all body tissues, concentrated in the liver and fat, rapidly metabolized, and excreted in the feces with a half-life of approximately 1 day. 245 was extremely persistent with less than 3% of the dose being excreted in the feces in 21 days. 245 redistributed from the body tissues to be stored primarily in adipose tissue. For both compounds, the radioactivity excreted was primarily metabolites of HCBs. By day 1, more of the tissue radioactivity in the old animals was metabolite than in young rats, suggesting slower elimination of metabolites in the senescent rats. Tissue/blood ratios were also decreased in the old relative to the young animals. Although tissue half-lives did not show any age-related change, the pool sizes increased for both compounds in the old animals. In all cases, the effects of aging seemed to be more pronounced upon the disposition of 245 than of 236. Thus, senescence may differentially affect the distribution, metabolism and excretion of different PCBs.

Chemical Disposition under the National Toxicology Program: Purpose and Function.

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Chemical disposition under the National Toxicology Program is designed to provide both applied knowledge to facilitate the choice of optimum doses to be used in NTP bioassays and basic knowledge on those chemical structure and property relationships which determine chemical disposition. The ultimate goal of each study of chemical disposition is to improve the extrapolation of laboratory data to man. This work is achieved through contract, interagency and inhouse research programs. Most studies utilize the adult male rat and radiolabeled chemicals, but studies may be designed to utilize most laboratory species, unlabeled compounds and any anticipated route of human exposure as the need arises. Individual studies have been designed to address chemical classes such as the benzidine- and benzidine congener-based dyes, single chemicals in several species such as TCDF or chemical types such as highly insoluble organics. However, most studies are designed to study the absorption, distribution, metabolism and excretion of individual chemicals. Nomination of chemicals, chemical classes or chemical types to be the subjects of chemical disposition studies is open to all interested individuals both within and outside the NTP.

An *in Vitro* Approach for Studying the Organ and Species Specificities of Chemical Carcinogen/Mutagen Activation.

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Cell structure techniques are being developed in an attempt to study the organ specificity of chemical carcinogen metabolic activation to genotoxic intermediates. Primary liver, lung, kidney and/or bladder cells from rat, hamster and/or bovine have been used for metabolic activation. Mutation and sister chromatid exchange (SCE) induction in Chinese hamster V79 cells and mutation of *Salmonella typhimurium* were the genetic endpoints measured. Rat liver, lung, bladder and kidney cells showed different metabolic capabilities in the activation of benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, 2-acetylaminofluorene, aflatoxin B_1 and dimethylnitrosamine. For 2-acetylaminofluorene, SCE induction and mutation of *S. typhimurium* were found to be more sensitive endpoints than V79 cell mutagenesis. Therefore when developing techniques to study the relative abilities of liver and bladder cells to activate several aromatic amines, *S. typhimurium* was used as the endpoint. Also, to insure sufficient bladder cells for conducting the experiments, methodologies for obtaining and utilizing liver and bladder cells from bovine were developed. Bovine bladder cells were more active than liver cells in activating the carcinogens aminofluorene, 2-acetylaminofluorene, benzidine, 4-aminobiphenyl and 2-naphthylamine to mutagens for *S. typhimurium.* The noncarcinogen, 1-naphthylamine, was not mutagenic with either cell type. The data show that the relative metabolic activation abilities of cells from various organs and/or species can be studied by the cell-mediated approach.

Fine Structure Analysis of Retroviral Long Terminal Repeats: Implications for Induced Gene Transpositions in Mammalian Cells.

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A very important question in genetic toxicology is the role that mobile genetic elements play in mutagenesis and carcinogenesis. Structure and sequence analysis has recently drawn attention to the parallels between the proviral structure of retroviruses and bacterial and eukaryotic transposable elements. A prominent feature of retroviruses is the long terminal repeat (LTR), which is structurally analogous to bacterial insertion sequences and which is thought to play a similar role in integration. In addition, this region contains sequences that are common to eukaryotic genes and appear to be involved in regulation of gene expression.

Retroviruses are known to be involved in carcinogenesis through recombination with other retroviral genes, and normal cellular genes (proto-oncogenes) and also by "downstream promotion" of cellular genes at novel integration sites. We have therefore investigated the possibility that endogenous proviruses act as transposons and may be targets for genotoxic agents. A model system of particular interest is the RFM/Un mouse radiogenic myeloid leukemia. This strain has a single

endogenous ecotropic retrovirus (RFV) that is expressed spontaneously in certain hematopoietic tissues throughout the animal's life. The strain is unique in expressing a strong inhibition that blocks virus infection and is therefore potentially an important model for studying intragenomic transposition of proviral sequences.

We have molecularly cloned and analyzed the RFV genome that was chemically induced from cultured fibroblasts. The restriction endonuclease map indicates that this virus is indistinguishable from the other murine endogenous ecotropic retroviruses. The LTR region has been sequenced, and the structural features characteristic of transposons were evident.

A small region of the *env* gene has been subcloned and used as a hybridization probe that specifically recognizes the ecotropic provirus. Comparison of normal and leukemic tissues by restriction enzyme digestion and hybridization with the "eco-specific" probe reveals that novel proviral integrations are found in the leukemias which may have arisen by transposition. The proximity of these sequences to activated *onc* genes and the expression of such genes by the proviral LTR are currently under study. Fine structure anlaysis of a population of viral LTRS reveals a sequence variability in a region that has been implicated in gene expression. The biological consequences of these variants are under study. A better understanding of the structure/function relationship of the LTR may come from further study of these variants.

The Role of Mitotically Defined Repair Functions in Meiosis. MICHAEL A. RESNICK and TERRY CHOW, *Toxicology Research and Testing Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709,* JOHN NITISS, *NIEHS and Illinois Institute of Technology, Chicago, IL 60616,* PETER MOORE, *NIEHS and University of Chicago, Chicago, IL 60637* and JOHN GAME, *University of California at Berkeley, Berkeley, CA 94720.*

Several genes identified with DNA repair functions in mitotic cells in the yeast *Saccharomyces cerevisiae* have been examined for their role in meiosis. Representatives of excision repair (*rad1*), mutagenesis (*rad6*) and double-strand break repair (*rad52*) pathways are able to undergo premeiotic DNA synthesis. Since *rad1*mutants exhibit normal meiosis while others lack to varying degrees recombination, viability and sporulation, some dispensable (in the absence of DNA damage) mitotic repair functions are essential in meiosis and others become essential only when cells are exposed to DNA damage.

The *RAD52* gene product becomes essential beginning with premeiotic DNA synthesis as evidenced by a concommitant loss of survival in *rad52* mutants. Coincident with loss in survival is the appearance of single-strand interruptions (SSI) in parental and newly synthesized DNA. Since at comparable times the *RAD+* cells exhibit a commitment to recombination that is absent in *rad52* mutants, the gene product is concluded to be essential in intermediate stages of recombination.

Alkaline single-strand deoxyribonuclease (SS-DNAse) has been examined in extracts from mitotic and meiotic cells of wild-type and *rad52* mutants. No noticeable difference is observed in total alkaline SS-DNAse level between logarithmically growing cells of these strains. However, differences are observed when rabbit antiserum raised against a purified SS-DNA-binding endoexonuclease from *Neurospora crassa* (a nuclease implicated in recombination and/or repair) is added to extracts. Of the alkaline SS-DNAse activity in wild-type extracts, 30-40% exhibits immunocrossreactivity; in contrast, no immunocrossreactivity is observed with the extracts of *rad52* strains.

Unlike the *RAD52* gene product, *RAD1* is essentialy during meiosis only when cells are UV-irradiated. No other excision repair mechanism is detected during meiosis. In the absence of *RAD1,* cells can tolerate a limited amount of DNA damage at early stages of meiosis due to the ability to synthesize DNA past pyrimidine dimers. However, the DNA damage affects recombination; a dose of 4 J/m^2 at the beginning of meiosis results in a 2 to 3-fold depression in meiotic intragenic recombination; intergenic recombination is nearly abolished. These results indicate that meiotic intragenic and intergenic recombination can be separated by UV at the beginning of meiosis and that the lack of intergenic recombination and presumably normal disjunction can account for the death of cells at late stages of meiosis.

The observation that DNA synthesis occurs past DNA damage within cells is being pursued to determine the nature of the synthesis. Extracts of cells are being examined for their ability to synthesize DNA *in vitro* on normal and damaged DNA templates; coding properties, if any, of the damage are also being examined. We have shown that cell extracts allow synthesis up to pyrimidine dimers. We are now attempting to determine whether there are conditions which will allow synthesis past dimers and whether extracts from cells at various times during meiosis will permit such synthesis.

Evaluation of Genetic Toxicity Assay Results on Twenty Chemicals: Implications for Animal Assays. JUDSON W. SPALDING and RAYMOND W. TENNANT, *Cellular and Genetic Toxicology Branch, Toxicology Research and Testing Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Twenty chemicals were tested for genetic toxicity in five classes of *in vitro* short-term tests. These tests represent a broad range of potential mechanisms of genetic toxicity and reflect the ability of chemicals to directly damage DNA or to alter gene expression. The specific tests selected were chosen because the protocols have received some form of previous evaluation or validation. The chemicals represent a wide range of structural classes and were tested and evaluated as coded compounds. The short-term tests used were: class I, microbial mutagenesis, the Salmonella/microsome (SAL) test; class II, mammalian cell mutagenesis, the (L5178Y) mouse lymphoma cell TK$^{+/-}$ forward mutation assay; class III, chromosome damage in mammalian cells, *in vitro* cytogenetics assay, in Chinese hamster ovary cells for the detection of chromosomal aberrations (CA) and sister chromatid exchange (SCE); class IV, mammalian cell transformation: the chemical enhancement of DNA virus transformation of Syrian golden hamster embryo cells by Simian adenovirus SA-7 (SHE-SA7); and the Balb/C 3T3 *in vitro* transformation assay; and class V, DNA damage/repair: the *in vitro* unscheduled DNA synthesis (UDS) in rat liver primary cell culture assay; and the *in vivo-in vitro* unscheduled DNA synthesis (HA-UDS) in rat liver primary cell culture assay. In the SAL, *in vitro* cytogenetics and L5178Y assays, chemicals were tested both with and without the presence of an exogenous metabolic activation (S-9) system. The S-9 fraction was prepared from liver homogenates of Aroclor 1254 induced male rats. In addition, the SAL test was performed with S-9 from Aroclor 1254 induced male Syrian hamsters. Concurrent positive and solvent or medium controls were performed in each test.

Interactions of 1,2-Dibromo-3-chloropropane with the Male Reproductive System. ARNOLD GREENWELL, FRANK HARRINGTON, BHOLA GUPTA, ROBERT MARONPOT, JAMES C. LAMB, IV and WILLIAM KLUWE, *Toxicology Research and Testing Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The nematocide 1,2-dibromo-3-chloropropane (DBCP) is a nephrotoxicant and gonadotoxicant in several mammalian species and has recently been shown to cause testicular atrophy and infertility in male pesticide formulators. Further toxicity studies were conducted in male, Fischer 344 rats to characterize the nature of the sterilizing effect, including gonadal sites of injury, age- and dose-response relationships, chronicity, and potential reversibility.

Single subcutaneous exposures to 40-100 mg/kg DBCP caused progressive testicular atrophy secondary to seminiferous tubular degeneration. Sperm concentration in the cauda (tail) epididymis was reduced in a temporal fashion commensurate with the testicular atrophy. A rapidly reversed vacuolar degeneration of the tubular epithelium was detected in the initial segment of the caput (head) epididymis. Moderate to severe testicular atrophy appeared to be essentially irreversible. Prolonged (70 day) oral studies indicated little cumulative toxic potential for DBCP.

Male rats treated at 6 days of age were equisensitive to adults with regard to the lethal potency of DBCP, but more susceptible to the gonadal effects, as indicated by irreversible testicular injury at doses as low as 20 mg/kg. Twenty-four day old rats were less susceptible to both the lethal and the gonadotoxic effects of DBCP than were adults.

Infertility occurred in male rats almost immediately after a single DBCP exposure, in contrast to a lack of effect on sperm concentration until much later. This effect appears to be due to an inhibition of sperm energy metabolism, as the ability of sperm to metabolize glucose to CO_2 was reduced by DBCP both *in vivo* and *in vitro*.

These results indicate that DBCP may cause sterility by two mechanisms: an immediate inhibition of sperm energy metabolism and a progressive testicular atrophy secondary to seminiferous tubular degeneration.

Renal Function Tests and Chemical Nephropathy. FRANK HARRINGTON, ARNOLD GREENWELL and WILLIAM KLUWE, *Toxicology Research and Testing Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Urinalyses and other noninvasive renal function tests have obvious advantages over invasive tests, such as direct morphological examination. Noninvasive tests can be performed sequentially on the same subjects, allowing for an evaluation of the development, progression and repair of kidney lesions. Moreover, functional assessments may be more realistic indices of nephropathy than microscopic aberrations. The quantitative urinary excretions of fluid, proteins, glucose, electrolytes, creatinine, renal cell enzymes and other substances in rats were monitored sequentially after single or repeated treatments with nephrotoxicants such as mercuric chloride, halogenated hydrocarbons or biphenyl. The results were compared with morphological changes in the kidney. The quantitative urinalyses were more sensitive and accurate indicators of nephropathy than were strictly qualitative procedures, and urinalyses or other renal function tests, in general, were more sensitive indicators of chemical insult than were morphologic examinations. Microscopic examination of the kidney, however, was useful in identifying the target cells within the kidney and often detected residual morphologic abnormalities despite return of renal function to normal. It is recommended that urinalyses or other noninvasive kidney function tests be used to monitor nephropathy during chemical treatment and recovery, and that morphologic examinations of the kidneys be conducted at termination to detect the site(s) of injury and possible residual effects.

Effects of Dimethyl Methyl Phosphonate on the Reproductive System of the Male Fischer 344 Rat. JUNE K. DUNNICK, BHOLA N. GUPTA, MARTHA W. HARRIS and JAMES C. LAMB, IV. *Toxicology Research and Testing Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Dimethyl methyl phosphonate (DMMP), a nerve gas simulant, has been used by the U.S. Armed Forces in a variety of experimental situations. The National Toxicology Program was asked to investigate the toxic properties of this substance and this study was conducted to determine reproductive toxicity. DMMP was administered to male Fischer 344 rats by gavage for 90 days at doses of 0, 250, 500, 1000 and 2000 mg/kg. At day 84 the rats were mated to untreated female Fischer 344 rats. There was a dose-related decrease in sperm count, sperm motility and the male fertility index. The male fertility index was 70%, 75%, 60%, 40% and 0% in the 0, 250, 500, 1000 and 2000 mg/kg dose groups. DMMP acted like a dominant lethal mutagen as demonstrated by an increase in the number of resorptions with increasing doses of the drug. The percent resorptions in the control group was 6.1% and increased to 14.9%, 37.8% and 79.1% in the 250, 500, 1000 mg/kg groups, respectively.

The testes of the male rat were examined histologically to determine the relationship between reproductive function and pathologic abnormalities. DMMP altered reproductive function at all dose levels, while histologic abnormalities were seen only in the high dose group. Changes in the testes of the high dose animals were characterized by lack of spermatogenesis or by degeneration, vacuolization and necrosis of spermatogenic cells. Histopathologic abnormalities of the kidney were seen in some animals from each of the dosed groups and microscopic changes of the epididymis and prostate were seen in some of the high dose animals.

Dimethyl methyl phosphonate is a representative of the organophosphorus chemicals, a class of compounds generally known for their neurotoxic properties. This particular organophosphorus compound had marked toxicity for the reproductive system of the male rat, toxicity that was not accompanied by overwhelming clinical or neurological toxicity. Such compounds may pose an unrecognized hazard in our environment.

Comprehensive Safety Evaluation of Di(2-ethylhexyl) Phthalate and Other Phthalate Esters by the National Toxicology Program. WILLIAM M. KLUWE, *Toxicology Research and Testing Program, National Institute of Environ-*

mental Health Sciences, Research Triangle Park, NC 27709.

Chronic toxicity testing of di(2-ethylhexyl) phthalate (DEHP) by the National Toxicology Program (NTP) has revealed hepatocarcinogenic effects in both rats and mice. Estimates of current U.S. production of DEHP, the major plasticizer for poly(vinyl chloride) products, are in excess of 2×10^8 kg/ yr. At a conference convened by the NTP and the Interagency Regulatory Liaison Group (IRLG) to discuss these and other relevant data on health aspects of phthalate ester plasticizers, it became clear that a number of interested groups were performing or considering research or testing programs concerning the phthalates. The NTP has prepared and is currently conducting the program designated below as an initial step in the comprehensive safety evaluation of phthalate esters and related compounds: genetic toxicity: mutagenicity, clastogenicity, DNA damage; tumor promoting abilities: liver and skin models; teratology: in-depth analysis for DEHP, screens for others; comparative dermal absorption: structure/ activity relationships; testicular toxicity of DEHP; carcinogenicity testing: diallyl phthalate, butyl benzyl phthalate, diethyl phthalate; hepatic effects of DEHP; hypolipidemic effects of DEHP.

The major objectives of this program are to characterize the toxic effects and risks associated with DEHP exposure, and to screen other phthalates for toxic potential.

Transposable Elements in Mendelian Populations. I. A. Theory. CHARLES H. LANGLEY and JOHN F. Y. BROOKFIELD, *Laboratory of Animal Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709,* and NORMAN KAPLAN, *Biometry and Risk Assessment Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Transposable elements are DNA sequences, found throughout eukaryotes, that transpose replicatively and cause numerous genetic and developmental effects on their hosts. A model of the evolution of transposable elements in Mendelian populations is proposed. From its analysis, formulae for the mean copy number and frequency spectrum are obtained.

Protein Structure and cDNA Cloning of Mammalian Lactate Dehydrogenase Isozymes A_4 (Muscle), B_4 (Heart) and C_4 (Testis). S. S.-L. LI, Y. -C. E. PAN, F. S. SHAREIF, K. AKAI, M. OKABE, M. SHIMIZU, H. F. TIANO, R. J. FELDMANN, W. M. FITCH and R. A. JUNGMAN, *Laboratory of Animal Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The amino acid sequences of lactate dehydrogenase isozymes (LDH-X; subunit C) specified to the testes of the mouse and rat have been determined. Of the 330 residues, 10% differ between the two LDH-X isozymes; most of these changes can be accounted for by single nucleotide changes. Two-thirds of the differing residues are located on the surface of the molecules and may be involved in the antigenic determinants unique to the LDH-X isozymes. A computer graphic system has been used to visualize the three-dimensional structure of the molecules and establish atomic relationships related to the amino acid sequence. The LDH-X isozymes are used by other groups at NIEHS as the basis for detecting mutations in single sperm cells. Mutations that modify the mouse LDH-X so that it is recognized by antibodies to the rat enzyme can be detected by fluorescence microscopy. The development of this mutation monitoring scheme depends upon knowing the three-dimensional structure of the isozymes and the types of mutational changes that can be detected antigenically.

Lactate dehydrogenase isozymes from mouse muscle (subunit A) and heart (subunit B) have also been studied, along with those from human heart, bovine heart, rabbit muscle and horse muscle. The tryptic peptide maps, amino acid compositions and partial sequences have been determined, and it is evident that the muscle and heart subunits show a closer relationship to each other than to the testis specific subunit. Since the evolutionary relationships among the subunits and among species groups are emerging, we may be better able to understand the types and amounts of genetic diversity that exist and how this diversity has played a role in the evolutionary process.

Amino acid sequence data provide a direct approach to the cloning of the LDH genes using recombinant DNA techniques. From the amino acid sequence, a short nucleotide sequence (approximately 14 nucleotides) that is not redundant and is unique to the gene encoding a specific LDH isozyme has been synthesized. This oligonucleotide is being used to identify the cloned recombinant DNA. Several cDNA clones have been isolated and the partial nucleotide sequences determined. Analysis of the DNA sequence encoding the protein and those flanking the structural gene is expected to yield important information about the gene's regulatory signals and to allow evaluation of mutational events that perturb the regulatory mechanisms as well as those that modify the protein coding sequence itself.

Human Mitochondrial DNA. C. F. AQUADRO and B. D. GREENBERG, *Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709,* and N. KAPLAN and K. RISKO, *Biometry and Risk Assessment Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

We have analyzed nucleotide sequence variation in an approximately 900 base pair region of the human mitochondrial DNA molecule encompassing the heavy-strand origin of replication and the D-loop. Our analysis has focused on nucleotide sequences available from seven humans. We have detected a high frequency of multiple (repeated) nucleotide substitutions and insertions/deletions. Two substitution biases are apparent, one favoring transitions by a factor of 32:1 over transversions, and the other favoring a high rate of turnover of purines relative to pyrimidines on the heavy strand of the mitchondrial DNA. The occurrence of these biases in coding and noncoding regions as well as rRNA and tRNA genes suggests that these phenomena may result from biases in the mutational pathways. In addition, the rate of substitution is significantly nonuniform throughout the D-loop region suggesting differing selective constraints or susceptability to mutation in various parts of this noncoding region. We have also modeled the dynamics of the substitution process. The results support the hypothesis that while a portion of the mitochondrial DNA molecule is relatively free to change at a very rapid rate, the

majority of the molecule is under very strong selective constraints as to base substitutions and other types of sequence alterations.

Insertion and Deletion Variation in Drosophila. C. F. AQUADRO, M. BLAND, C. H. LANGLEY, E.A. MONTGOMERY, and W. F. QUATTLEBAUM, *Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Restriction map variation has been determined in a 12,000 base pair region around the alcohol dehydrogenase structural gene (Adh) in 48 lines of *D. melanogaster* from several geographically distinct populations and several closely related species. The most striking result is the high level of insertion and deletion variation within species, and the low level between species. Significantly, no insertion/deletion variation occurred within the Adh coding block. These observations, together with the geographically restricted distribution of individual insertions or deletions, in the face of evidence for free gene exchange between localities, suggest many of these variations are deleterious. In addition, we have evidence that most of the large inserts are transposable elements and that at least one is correlated, if not responsible for, an altered level and pattern of expression of the Adh gene. We have also screened the same 48 lines for variation in a 40,000 base pair region around the dopa decarboxylase (Ddc) gene. The preliminary impression for this region is quite different from Adh, in that there are fewer insertions and deletions. However, one of them occurs within the Ddc transcript.

Cloning and Genetic Analysis of Hybrid Dysgenesis-Induced Alleles at an RNA Polymerase II locus in *Drosophila melanogaster*. ROBERT A. VOELKER, S. M. HUANG, H. GYURKOVICS, G. B. WISELY, and PAUL M. BINGHAM, *Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709,* and LILLIE L. SEARLES, ROBERT S. JOKERST and ARNO L. GREENLEAF, *Duke University, Durham, NC 27710.*

The L5 locus, an allele of which (RpIIC4) confers α-amanitin resistance on RNA polymerase II of *Drosophila melanogaster,* maps at division 10C on the polytene X-chromosome and is lethal-mutable. Lethal alleles of this locus were generated by hybrid dysgenesis, a phenomenon in which a mobile DNA sequence known as the P-factor is mobilized and caused to insert at numerous places throughout the genome. One of these lethal alleles was shown by *in situ* hybridization to have a P-factor insertion in the 10C region (as well as other insertions at other sites). Probable causality between the P-factor insertion and the resulting lethality was indicated by the observation that in several independent reversions of the lethal the P-factor insertion at 10C was lost. A whole-genome charon-30 phage library of P-factor-containing mutant DNA was constructed and screened with P-factor. DNAs from the P-factor-containing phage were then hybridized *in situ* to wild type polytene chromosomes to identify those phages which contained unique sequences from the 10C region. One phage was identified which carried 11 kb of Drosophila DNA which included a 1.3 kb P-factor insert near one end. Subcloned fragments of the DNA were used to recover clones containing nearly 25 kb of DNA from wild type libraries. Subclones of this region were used to identify six different polyadenylated messages produced by this region; one (ca. 7 kb) of these messages has been identified as that encoding the α-amanitin resistance-conferring subunit. Efforts are being made to correlate the other polyadenlyated messages with other genetically detected functions in the region.

Mutation Detection in Mice. F. M. JOHNSON, S. E. LEWIS and L. C. SKOW, *Mammalian Mutagenesis Section, Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709* and D. P. LOVELL, *MRC Laboratory Animals Centre, Carshalton Beeches, Surrey, England.*

Heritable alterations in DNA and chromosomes may be induced as a result of exposure to certain agents in the environment. We are interested in applying various techniques for the purpose of identifying induced and spontaneous mutations in mice that can in turn serve as a basis for understanding the biological consequences of and biochemical phenomena involved in mutational alterations in man.

Techniques being investigated include electrophoresis, isoelectric focusing, and morphological/anatomical examinations of eye, skeleton, and other regions of the body. The methods have provided for the identification of abundant variation. Using classical genetic approaches, variation is investigated to determine if it is attributable to heritable factors. When heritable factors are involved, studies are conducted to determine their origin, i.e., newly induced mutation, newly arisen spontaneous mutation, or genetic variation originating in the past. Induced mutations are produced by exposing mice to mutagenic agents and by subsequently examining parents, F_1 offspring, and later generations for the characteristics of interest.

Having available a number of mutations in specific gene products makes it possible to explore many questions of fundamental and practical importance. Thus, one can utilize genetic errors to better understand normal genetic organization and regulation, as well as a wide range of functions subject to genetic control. Furthermore, with mutagen exposures of mice it may be possible to provide models of particular genetic diseases that are not now available in mice or other experimental mammals. Such animal models are useful for investigating techniques that may ultimately cure or lessen the severity of genetic diseases in man.

The degree to which human populations may be subject to an increased burden of genetic disorders as a result of increased mutation rates is presently uncertain. When mice are exposed to measured doses of specific mutagens, the frequency and type of various mutational events can often be determined. By combining such data with information on the functional effects associated with the mutations in homozygous and heterozygous condition, it may be possible to gain improved understanding of a potentially serious health problem.

Mutagenesis Associated with Bacterial Conjugation. B. A. KUNZ and B. W. GLICKMAN, *Laboratory of Molecular Genetics, National Institute*

of Environmental Health Sciences, Research Triangle Park, NC 27709.

A single DNA strand of the F plasmid is passed from donor to recipient during bacterial conjugation. DNA synthesis produces complementary strands using the transferred and retained strands as templates. This conjugal DNA synthesis occurs in cells that have been heavily irradiated to abolish vegetative replication. Thus, conjugal DNA synthesis appears able to circumvent blocks to vegetative DNA replication. A mechanism that enables DNA synthesis to bypass template damage might be expected to alter the fidelity of DNA replication. To test the fidelity of conjugal DNA synthesis we employed the *E. coli lacI* system which permits the detection of nonsense mutations at 65 independent sites within the *lacI* gene. Knowledge of the DNA sequence and location of the nonsense mutations allows one to correlate each mutation with a specific base change. In this system the *lacI* gene is situated on the F′ plasmid carried by donor cells and the homologous chromosomal gene is deleted in donors and recipients. For our study, *lacI* mutations were selected subsequent to conjugal transfer of the F′. We found that the frequency of *lacI* nonsense mutations is 10-fold higher after conjugation than in the absence of transfer. Mutational spectra revealed that this increase is due to both transitions and transversions. Moreover, *recA*-dependent error-prone processes appear not to be responsible for the increase in mutation. These results suggest that conjugal DNA synthesis may be inherently less accurate than vegetative DNA replication. Alternatively, spontaneous damage occurring in F′ DNA may not be repaired once conjugation is initiated, leading to fixation of mutations during conjugal DNA synthesis and so to the enhanced recovery of nonsense mutations.

The Role of DNA Secondary Structure in Mutation. L. S. RIPLEY and B. W. GLICKMAN, *Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709*

Palindromic sequences provide inherent self-complementarity and hence permit the formation of unusual DNA structural intermediates. A general model based on the processing of these intermediates predicts a wide range of mutational outcomes in both quasipalindromic and palindromic DNA sequences.

In quasipalindromic sequences, self-complementarity is not perfect, and processing of imperfectly paired intermediates leads to more perfect palindromes and hence the production of base substitutions, additions, deletions or inversions. For example, production of these mutations may be viewed as a mismatch correction event within a hairpin structure. The unpaired bases within the hairpin in the noncomplementary portions of the quasipalindrome may either be removed or serve as templates for the insertion of DNA bases.

In both quasiplaindromic and palindromic DNA sequences, deletion and duplication events are predicted outcomes of the formation of aberrant DNA structures. Within these structures, normally distant base pairs are brought into immediate proximity and eventually define the end points of deletion or duplication mutations.

Di-2-ethylhexyl Phthalate as a DNA Precursor. PHILLIP W. ALBRO, *Laboratory of Environmental Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Feeding di-(2-ethylhexyl) phthalate (DEHP) at a very high level in the diet continuously for two years reportedly caused an uncreased incidence of hepatocarcinoma in rats and mice. Radioactivity from C-1 of the ethylhexyl portion of DEHP or of the corresponding monoester (MEHP) becomes covalently associated with liver DNA *in vivo*. The present studies indicate that the phthalate portion of DEHP or MEHP does not bind to liver DNA *in vivo*. Moreover, C-1 of the ethylhexyl portion associates with DNA, apparently because it is a metabolic precursor of carbamyl phosphate, in turn a precursor of normal DNA bases. No evidence for the formation of a DNA–ethylhexanol adduct could be obtained. However, the possible association of any or all of the other seven carbon atoms of the ethylhexyl unit with DNA remains to be investigated.

Application of High-Performance Liquid Chromatography to the Analysis of Peptide Hormones. O. HERNANDEZ, K. DERMOTT and L. H. LAZARUS, *Laboratory of Environmental Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The application of reversed-phase high-pressure liquid chromatography (RP-HPLC) to the isolation and purification of unprotected peptides is a well established technique. The combined use of RP-HPLC and radio-immunoassay (RIA) has evolved as a powerful tool for the identification of peptides in tissues. The potential crossreactivity problems inherent to the RIA techniques are virtually eliminated when coupled to the high resolving power of RP-HPLC. This latter analytical procedure discriminates between structurally related peptides differing by a single amino acid residue or even stereoisomers. The combination of these two procedures provides a selective and highly sensitive—quantitative at the picogram level—method for the identification of peptides. A requirement for compatibility is the use of volatile buffers in RP-HPLC to avoid interferences in the RIA.

An active area of research is the mechanisms(s) by which peptides are separated under RP-HPLC conditions. The information available underscores the importance of hydrophobic interactions between the peptide and the RP-HPLC column. Empirical correlations have been developed in which the retention time of a peptide in RP-HPLC may be predicted based on the sum of the hydrophobic constant values for the amino acid residues present. By utilizing these observations we explored ways of enhancing the information available from routine RP-HPLC experiments. Our approach was based on the premise that the retention of a peptide in a RP-HPLC column is determined by the extent of hydrophobic binding. If it were possible to modify nondestructively, the intrinsic hydrophobicity of the molecule this would be reflected in shorter or longer retention times depending on the nature of this modification. Because of our interest in bombesin our initial efforts were focused on this peptide. On a first attempt we decided to use pH as a variable to alter the ionic character of the peptide molecule and hence the hydrophobic surface available for binding to the RP-HPLC column. By observing the behavior of bombesin under acid (pH 4) and neutral (pH 7) conditions the extent of ionization of the histidine residue at position 11 would determine the ionic nature of this peptide. Other peptides with and without histidine residues were also

examined. The solvent strength was maintained constant so as to isolate the effect of pH. Isocratic chromatographic conditions were selected for expediency and to avoid pH fluctuations due to increasing organic modifer concentrations as found under gradient conditions. The resulting methods provide diagnostic procedures for the identification of bombesin and have potential application for rapid clinical analysis of tissue samples.

Application of Fast Atom Bombardment Mass Spectrometry to the Analysis of Biologically Important Molecules. D. J. HARVAN, H. J. WALTHER and J. R. HASS, *Laboratory of Environmental Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Of the many possible mechanisms for chemical carcinogenesis, the formation of covalent bonds between the carcinogen and some part of DNA is likely an important step. The study of such adduct formation has been limited by the experimental difficulties associated with the identification and quantitation of such compounds. The recent development of ionization techniques which allow the analysis of highly polar compounds by mass spectrometry opens new possibilities for the study of DNA constituents. In this paper is described the results of applying one of these (fast atom bombardment, FAB) to low molecular weight polynucleotides.

Sample volalitilization and ionization results from interaction with an energetic beam of rare gas atoms which are produced by resonance charge exchange neutralization of an appropriate rare gas ion beam which is formed in an electrical discharge. It is important to have the sample dissolved in a suitably viscous, polar material, such as glycerol. The mechanism of ionization is poorly understood at present, but intense, stable ion beams which give molecular weight and fragmentation reaction information result.

Some of the samples that have been ionized by this method have been methyl benzanthracenes bound to various nucleosides, underivatized di- and tri- nucleotides, and underivatized peptides. The method usually gives rise to ions corresponding to $[M + H]^+$, $[M + \text{sodium}]^+$, and $[M + \text{glycerol}]^+$. Some fragmentations of the samples are often observed, and may provide structural information. Spectral interpretation is simplified by means of B/E linked scanning of the mass spectrometer to elucidate fragmentations of selected ion species.

The technique has proven to be quite useful for the ionization of previously difficult biological samples. The sample requirements are in the range of 10^{-8}-10^{-6} g for scanned mass spectra. Future experiments involve the study of higher molecular weight species, and different types of bombarding reagents.

Amphibian Peptides in Health and Disease, LAWRENCE H. LAZARUS, OSCAR HERNANDEZ, GLORIA D. JAHNKE, MICHAEL D. ERISMAN, RICHARD P. DIAUGUSTINE, CATHY M. SOLDATO, WILLIAM E. WILSON, and LAURA J. STONE, *Peptide Neurochemistry Workgroup, Laboratory of Behavioral and Neurological Toxicology, and Laboratory of Environmental Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Small-cell carcinoma of the lung is a highly lethal and metastatic neoplasm in humans which can be attributed to the effect of environmental carcinogens, such as those found in cigarette smoking. Frequently, patients with this disease have elevated levels of certain peptide hormones in their blood which are produced and secreted by the tumor. To this growing list of tumor-associated hormones, we recently discovered the presence of both amphibian peptides bombesin and physalaemin in the tumor grown in athymic (nude) mice. This discovery was confirmed by two other laboratories and bombesin is now being touted by the medical community as a potential peptide marker for this type of tumor. Through a series of chemical modifications, we have identified the presence of the amino acids pyroglutamic acid, arginine, histidine and the amidated terminal residue as methionine in tumor bombesin as being the same in the amphibian peptide. Tumor physalaemin, like its amphibian homolog, contains pyroglutamic acid, lysine and amidated methionine.

Another molecular species of mammalian bombesin is currently being investigated by us in milk which normally contains a large array of hormones in addition to other nutrients. Its concentration in milk is sufficient to bring about a rise in hormone levels and gastric acid secretion in both neonates and adults. In fact, the hormones influence by the infusion of bombesin in humans and by the peroral consumption of milk in neonates are identical. Furthermore, the use of milk in ulcer therapy is now seriously questioned by investigators in San Diego due to the acid content in stomachs of ulcer patients drinking milk.

Chrysotile Asbestos Inhalation in Rats: Deposition Pattern and Reaction of Alveolar Epithelium and Pulmonary Macrophages. ARNOLD R. BRODY, LILA H. HILL, BERNARD ADKINS, JR. and ROBERT W. O'CONNOR, *Laboratory of Pulmonary Function and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The initial deposition and subsequent translocation of chrysotile asbestos were studied in the lungs of rats exposed for 1 hr in inhalation chambers. Using scanning and transmission electron microscopy of tissue fixed by vascular perfusion, we determined that the majority of fibers that pass through the conducting airways deposits at the bifurcations of alveolar ducts. The farther an alveolar duct bifurcation was from its terminal bronchiole, the less asbestos was observed. The amount of asbestos present on the alveolar duct surfaces was significantly decreased 5 h after cessation of the 1-hr exposure. Some fibers were taken up by Type I epithelial cells during the first hour of dusting, and this process continued through the 8-day period in which the animals were studied. As early as 24 hr after exposure, there was an accumulation of macrophages at the sites of initial asbestos deposition. This may be a significant cellular response in the early pathogenesis of asbestosis.

Interstitial Accumulation of Inhaled Chrysotile Asbestos Fibers and Consequent Formation of Microcalcifications. ARNOLD R. BRODY, and LILA H. HILL, *Laboratory of Pulmonary*

Function and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Previous studies have shown that inhaled chrysotile asbestos impacts initially at the bifurcations of alveolar ducts in the lungs of rats. Asbestos fibers are transported through alveolar epithelial cells at these bifurcation regions at the interstitium during the 24-hr period after a 1-hr exposure. To further these studies, white rats were exposed to an aerosol of chrysotile asbestos for 1 hr. Animals were sacrificed, and the lungs were fixed by vascular perfusion immediately after and 1 month after exposure. Blocks of tissue were prepared for light and electron microscopy. At 1 month after exposure, numerous asbestos fibers had accumulated within the lung interstitium at alveolar duct bifurcations. Many of these interstitial fibers were found in the center of intracellular microcalcifications. The presence of calcifications was proven by X-ray energy spectrometric analysis of the inclusions *in situ*. Clear X-ray peaks for calcium and phosphorus were demonstrated. It is proposed that 1 month after a 1-hr exposure to chrysotile asbestos, fiber-induced membrane injury in cells of the lung interstitium leads to formation of microcalcifications. This may represent the presence of early cell injury in the initial pathogenetic sequence of asbestosis.

Intra- and Extracellular Distribution of Surfactant Phospholipids in the Lungs of Rabbits.

L. A. DETHLOFF and G. E. R. HOOK, *Laboratory of Pulmonary Function and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Pulmonary surfactant phospholipids may be considered to exist within at least two anatomically distinct pools. The intracellular pool, located within Type II alveolar epithelial cells, is comprised of storage structures called lamellar bodies. The extracellular pool resides in the alveoli where surfactant prevents alveolar collapse by reducing surface tension at low lung volumes. Methods are presented for the isolation and quantitation of these two pools. Alveolar lavage was employed to obtain the extracellular pool, while the characteristic density of lamellar bodies was exploited to isolate the intracellular pool. As determined by thin-layer chromatography, the phospholipid compositions are quite similar and indicate that these pools may accurately represent intracellular and extracellular surfactant. Estimates for the intra- and extracellular surfactant pool sizes in the New Zealand White rabbit are 1.65 +/− 0.45 mg/g and 2.77 +/− 0.49 mg/g of lung tissue, respectively.

Oxidation of 2-Aminofluorene by Prostaglandin Endoperoxide Synthetase.

JEFF A. BOYD, DONALD J. HARVAN and THOMAS E. ELING, *Laboratory of Pulmonary Function and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

We have examined the prostaglandin endoperoxide synthetase (PES)-dependent metabolism of the arylamine carcinogen 2-aminofluorene (2-AF). Ram seminal vesicle microsomes fortified with arachidonic acid metabolize 2-AF to products covalently bound to microsomal macromolecules, water soluble metabolites, and two organic extractable metabolites. The organic extractable metabolites were identified by co-chromatography (HPLC), UV-VIS spectrophotometry, and mass spectrometry as 2-nitrofluorene and 2,2′-azobisfluorene. Hydrogen peroxide also supports 2-AF metabolism to the same products, suggesting that the hydroperoxidase activity of PES is responsible for the cooxidation. Total metabolism of 2-AF at 50 μM is 50%. Several experiments indicate that molecular oxygen is not utilized in the reaction. The highly reactive oxygenated metabolites of 2-AF, *N*-hydroxy-2-AF and 2-nitrosofluorene, are metabolized by PES to one major product, 2-nitrofluorene. The metabolism of 2-AF, *N*-hydroxy-2-AF, and 2-nitrosofluorene is extremely rapid, reaching completion in less than 30 sec. The horseradish peroxidase/H_2O_2 system also metabolizes 2-AF to 2-nitrofluorene and 2,2′-azobisfluorene. These results suggest that 2-AF is oxidized to an electrophilic intermediate by PES, presumably N-hydroxy-2-AF, 2-nitrosofluorene, and/or free radical intermediates which either bind to macromolecules or are rapidly further oxidized to 2-nitrofluorene and 2,2′-azobisfluorene.

Quantitative, Clonal Studies of Neoplastic Progression of Rat Tracheal Epithelial Cells.

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We have developed a system for quantitating early carcinogen-induced changes of rat tracheal epithelial (RTE) cells and their subsequent progression to neoplasia. Methods are described for growing normal RTE cells in culture and for selecting and quantitating the appearance of enhanced growth (EG) variants. Normal RTE cells were isolated by pronase treatment of Fischer 344 rat tracheas and grown on lethally irradiated 3T3 cells in Ham's F12 medium with 5% fetal calf serum, insulin (1 μg/mL), and hydrocortisone (0.1 μg/mL). Colony forming efficiencies were 5-10% for at least five passages (>20 population doublings). MNNG (0.1-0.6 μg/mL in Hepes buffered F12) treatment resulted in dose-dependent reductions in colony forming efficiencies. At 1 to 7 days after MNNG treatment, EG variants were selected by removing feeder cells and refeeding cultures with complete medium or by replating RTE cells onto dishes without feeders. After 4 weeks, large colonies of small hyperchromatic cells with increased nuclear: cytoplasmic ratios were observed. Frequencies of altered cells increased in a dose dependent manner yielding a maximum frequency of about 4% per surviving colony forming cell at doses of 0.3-0.6 μg MNNG/mL which gave relative survivals of 10-50%. Similar results were obtained with a variety of carcinogens including γ-radiation, B(a)P, B(a)P diol epoxide, and nickel salts. The induced frequencies of EG variants obtained were similar to those reported for the induction of morphological transformation of fibroblasts in culture. Control cultures of RTE cells yielded frequencies of EG variants of <0.1% per colony-forming cell.

Continued propagation of cultures of EG variants resulted in the accumulation of cells capable of growing in semisolid agarose medium. These colonies when isolated had high colony forming efficiencies in agarose medium (≈40%) and were nontumorigenic in nude mice. After extensive propagation *in vitro*, EG variants developed neoplastic potential and formed squamous cell carcinomas when injected into nude mice. This work provides a system for characterizing and quantitating the frequency of early carcinogen-induced changes and the development of neoplasia in respiratory epithelial cells.

Pulmonary Macrophages Exhibit Morphological, Functional and Biochemical Changes after One Hour of Exposure to Chrysotile Asbestos *in Vivo*. D. B. WARHEIT, I. Y. CHANG, I. H. HILL, and A. R. BRODY, *Laboratory of Pulmonary Function and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Chronic inhalation of asbestos clearly has been associated with interstitial fibrotic lung disease. The pulmonary macrophage is a cell-type which has been implicated in the development of this disease. Our *in vivo* data show that the earliest macrophage response to asbestos inhalation is the recruitment of these cells to the sites of original asbestos deposition with subsequent phagocytosis of these fibers. Accordingly, we are studying the effects of inhaled asbestos on macrophages recovered by lavage, using morphological, biochemical and functional approaches.

No significant differences were found in cell numbers and viabilities of macrophages recovered from asbestos-exposed and sham-exposed animals at 24 hr, 48 hr, and 8 days after a 1-hr exposure. Asbestos-exposed macrophage injury was quantified by ultrastructural assessment of surface morphology. Correlated studies of iron bead phagocytosis were carried out using backscattered electron imaging. We detected small but significant changes in the morphology and phagocytic capacity of asbestos-exposed macrophages at the earlier two time periods. Using biochemical measurements, we found increased intra- and extracellular levels of acid hydrolases in the cells of asbestos-exposed animals following a 24-hr culture period. These data correlate with cytochemical findings which show an increased degree of acid phosphatase staining in asbestos-exposed macrophages. Utilizing electron microscopic, X-ray analytic techniques established in our laboratory, we found that 25-30% of the lavaged, cultured macrophages contained asbestos fibers. Our data indicate that a brief, 1-hr exposure to chrysotile asbestos elicits subtle, but measurable changes in functional, morphological and biochemical parameters exhibited by pulmonary macrophages. These changes could signal the development of cellular alterations which play a role in the pathogenesis of asbestos-related lung disease.

Free Radical Formation by Hepatic Microsomal Cytochrome P-450. R. P. MASON, W. G. HARRELSON and C. MOTTLEY, *Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

In the past, studies of the anion radical metabolites formed by microsomal one-electron reduction implicated one-electron donation from NADPH-cytochrome P-450 reductase. Investigations of nitro, azo, and quinone anion radical metabolites formed by this enzyme have been described. NADPH-cytochrome P-450 reductase also reduces quinoneimines, such as actinomycin D and dichloroindophenol, to semiquinonimine free radicals. More recently, cytochrome P-450 has been found to transfer one electron to toxic chemicals such as carbon tetrachloride, gentian violet, sulfur dioxide and molecular oxygen.

When gentian violet is metabolized under a nitrogen atmosphere by rat hepatic microsomes supplemented with NADPH, a single line ESR spectrum is obtained. The g value of the gentian violet free radical metabolite was measured as 2.0028, which is in agreement with the reported value of the photochemically produced tri(*p*-dimethylaminophenyl)methyl radical. Either CO or metyrapone inhibits radical formation by 50%, implicating cytochrome P-450 involvement.

In an atmosphere of nitrogen, rat liver microsomal incubations containing bisulfite (aqueous sulfur dioxide) and NADPH also form a free radical with a single-line ESR spectrum (g = 2.0056). When the N_2 atmosphere was replaced by CO this ESR signal decreased by more than 90%. Metyrapone also totally suppresses the ESR signal. These results imply that cytochrome P-450 reduces bisulfite to the sulfur dioxide anion radical. Oxygen completely inhibited the formation of this radical as is consistent with oxygen being a competitive inhibitor for the reduced heme of cytochrome P-450.

We have used the spin trapping technique to demonstrate the microsomal reduction of oxygen to superoxide, which is also inhibited by CO and metyrapone. Whether cytochrome P-450 or its reductase will donate an electron to an electron-accepting xenobiotic can not be predicted *a priori*.

***In Vitro* Effects of Microwave Radiation on Rat Liver Mitochrondria.** M. J. GALVIN, M. S. DUTTON and D. I. MCREE, *Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Liver mitochondria were exposed *in vitro* at 30°C to microwave radiation (2.45 GHz) during the various states of respiration (resting, state 1; substrate-dependent, state 2; ADP-stimulated, state 3; and ADP-depleted, state 4). At 10 or 100 mW/g, with succinate as substrate, no effect was observed on the resting, respiration rates, 2, 3, or 4, or the respiratory control ratio (state 3/state 4). With glutamate as substrate, no effects occurred at 10 mW/g, however, 100 mW/g caused an increase in respiration states 2 and 4 and a decrease in the respiratory control ratio. In addition the influence of microwave exposure on the *in vitro* response of mitochondria to endotoxin was determined. Mitochondria were simultaneously exposed to comparable levels of microwave radiation and to endotoxin concentrations which caused a 50% reduction in ADP stimulated respiration (state 3). State 2, 3 and 4 respiratory rates were determined 5 min post treatment. At SAR's of 10 and 100 mW/g there was a potentiation of the endotoxin actions on the mitochondria which was exhibited by a complete rather than a 50% inhibition of state 3 respiration.

The results demonstrate that high levels (100 mW/g) of microwave radiation can affect certain aspects of mitochondrial function in the absence of measurable thermal gradients. In addition, levels of microwave radiation at an SAR as low as 10 mW/g, can act as a potentiators of biological stressors such as endotoxin.

Latent Effects of Microwave Radiation in Japanese Quail Exposed during Embryogeny. D. I. MCREE, M. J. GALVIN and J. P. THAXTON, *Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Fertile Japanese quail eggs were exposed to 2.45 GHz CW microwave radiation for the first 12 days of embryogeny to an incident power density of 5 mW/cm^2 (SAR = 4.03 mW/g). The

exposed and nonexposed chicks were 6 or 12 weeks of age for the hematological studies and 6, 12, or 22 weeks of age for the immunological studies. In the hematological studies, red blood cell (RBC) numbers, pack cell volumes (PCV), total hemoglobin levels, and mean cell volumes (MCV) were significantly greater in the exposed group for both the 6 and 12 old quail. Mean corpuscular hemoglobins and mean corpuscular hemoglobin concentrations were not changed. Hemorrhagic stress at 3-day intervals (3, 6 and 9 days) produced no effects different from that of the control quail. These results indicate that microwave exposure during embryogeny causes increased numbers of RBC in adult quail, but a normal erythropoietic ability following hemorrhage.

In the immunological studies, the quail were evaluated for humoral immune responsiveness to chuker red blood cells, delayed hypersensitivity to phytohemagglutinin, and total and absolute circulating levels of leucocytes. All parameters were equivalent in exposed and nonexposed controls at 6-weeks of age. However, in 12- and 22-week old quail, delayed hypersensitivity was reduced in exposed females. These data show that exposure of Japanese quail during embryogenesis reduced cell mediated immune potential and induced a general leucocytosis in females.

Ototoxicity of *Cis*-Dichlorodiammine Platinum (II) in Guinea Pigs. TERUZO KONISHI, *Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709,* and BHOLA N. GUPTA, *Toxicology Research and Testing Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Effects of *cis*-dichlorodiammine platinum (II), an agent with potent antineoplastic activity, were studied in guinea pigs. With multiple treatment with daily dose of 1.5 mg/kg of *cis*-dichlorodiammine platinum, the cochlear microphonics were suppressed. Suppression of the cochlear microphonics was greater in the basal turn than in the third turn of the cochlea. There was a close correlation between loss of the cochlear hair cells and suppression of the cochlear microphonics. The endocochlear potential was decreased in the basal turn but remained unchanged in the upper turn of the cochlea. The sodium, potassium and chloride concentrations in both endolymph and perilymph were not affected. Slight and moderate congestion and regeneration of tubular epithelium of kidney were observed in treated guinea pigs.

Electrochemical Profile for Potassium Ions across the Cochlear Hair-Cell Membranes of Normal and Noise-Exposed Guinea Pigs. T. KONISHI and A. N. SALT, *Laboratory of Experimental Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The electrochemical driving force for movement of K^+ ions across the hair cell membranes was determined in normal and noise-exposed guinea pigs. The measurement of the electrical potential and potentiometric determination of K^+ activity difference across the cell membranes were accomplished with double-barreled K^+-sensitive microelectrodes. The results suggested that the resting potential of hair cells was mainly generated by the K^+ diffusion across the basolateral membranes and contact of the apical membranes of hair cells with K^+-rich endolymph served as a mechano-electric transducer. Although the cochlear microphonics recorded extracellularly were severely suppressed in guinea pigs exposed to noise at 115 dBA for 7 days, the electrochemical profile for K^+ across the cell membranes of serviving hair cells did not show marked changes. The ratio of intracellular ac receptor potential to extracellular cochlear microphonics was much greater in surviving hair cells of noise exposed guinea pigs.

Identification of the Free Radicals Produced during the Photolysis of 4-*tert*-Butyl-3-methoxy-2,6-dinitrotoluene (Musk Ambrette). A. G. MOTTEN, C. F. CHIGNELL and R. P. MASON, *Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Musk ambrette, a common component of perfumes, soaps and some food flavorings, can cause cutaneous photosensitization reactions including photo-allergy. These may be mediated through free radicals formed during photolysis. When musk ambrette was photolyzed in basic methanol, two distinct nitro anion radicals were identified by electron spin resonance. One radical was centered on a nitro group in the plane of the aromatic ring, while the other was centered on a nitro group twisted out of the plane of the ring due to steric hindrance by bulky substituents on either side of the group; the two radicals appeared to interconvert and maintain an equilibrium concentration ratio. Two closely related compounds which are also used in perfumes, but have not been reported to cause photosensitizing reactions, also produced free radicals during photolysis. Musk xylene (2,4,6-trinitro-1,3-dimethyl-5-*tert*-butylbenzene) generated two nitro anion radicals, both of which were centered on twisted nitro groups, while musk ketone (3,5-dinitro-2,6-dimethyl-4-*tert*-butylacetophenone) produced only one nitro anion radical which is also twisted. Although these nitroanion radicals are probably the first step in the photolysis of these nitroaromatic molecules it seems likely that they will undergo further reduction to produce more reactive species including nitroso compounds.

Single Fiber Scheme for Measuring Physiological Membrane Motions, R. O. COOK and C. W. HAMM, *Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The dimensions (~ 100 μ in width) and restricted access (cannot be approached perpendicularly) to the basilar membrane have long frustrated attempts to perform a frequency analysis at the primary auditory system transduction site. Newly developed optic fiber fusion and etch technology allows light traveling in several fibers to be efficiently coupled into a single small (40-120 μm) fiber. This development, coupled with the fact that light can be forced to exit a fiber at 90° by cleaving the fiber at 45° and sputtering a reflective gold coating on the cleaved face, suggested that useful measures of membrane motion might be obtained by directing light from a single 50 μm fiber onto the vibrating membrane and measuring the differently reflected light at a photosensor. Even when extremely bright (2-200 W/cm^2) electroluminescent sources are used as illuminators, the small fiber size (~ 50 μm) and low reflectivity (~ 0.2%) of the basilar membrane result in

return of only ~ 10^{-7} W dc or less to the photosensor. By use of state-of-the-art electro-optic detection circuitry, < 1Å/ $\sqrt{Hz}$ resolution over a 20-kHz bandwidth has been demonstrated against reflective targets, and < 75 Å/ $\sqrt{Hz}$ from the third turn of the basilar membrane of a guinea pig cochlea.

Influence of 17-β Estradiol on Hepatic Protein Synthesis: Analysis by Two-Dimensional Gel Electrophoresis.

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In an attempt to more precisely characterize estrogen influences on hepatic function, we have employed two-dimensional gel electrophoresis to analyze the protein synthetic profile of liver cells. Our results have shown selective alterations in hepatic protein synthesis following estrogen treatment. In particular, several estrogen-dependent proteins (F1-F7) were observed in female liver. Following ovariectomy, the expression of proteins F1-F5 and F7 were significantly reduced, whereas the expression of protein F6 was enhanced. Treatment of ovariectomized animals with either 17ß-estradiol or the nonsteroidal estrogen DES was capable of partially restoring the protein synthetic profile. Comparison of hepatic protein profiles between males and females revealed several sex-related differences. Proteins M1-M10 were either not expressed in females or were present in much lower amounts relative to total protein synthesis. Furthermore, proteins F2 and F4 present in intact females were not observed in male liver cells. The general influence of either 17ß-estradiol or DES on male liver protein synthesis resulted in selective but marked suppressions of certain proteins (M1-M3 and M7-M10). Since estrogen receptors are present in both male and female livers, the results of our study suggest, in part, that modulation of protein synthesis for specific proteins could be mediated through a receptor mechanism.

Studies on the Mechanism of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Toxicity: Nutrient Assimilation.

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a highly toxic contaminant of organochlorine herbicides, produces a starvation syndrome in laboratory animals. Seven days after TCDD treatment (52 μg/kg, gavage) lipid assimilation is altered in adult Fisher rats. After a corn oil meal (1 mL gavage), large lipid droplets aggregate in the absorptive intestinal cells and remain longer than the lipid in control cells. Twenty-four hours later, small lipid droplets remain in the basal regions of the absorptive cells of the TCDD-treated rats. One week after TCDD treatment, the serum glucose levels were 1/2 to 1/3 of the control values, while serum triglyceride levels were 3 to 4 times higher than the control values. Transport of amino acid and monosaccharide, as determined by *in vitro* active transport of radiolabeled leucine and 3-O-methylglucose by isolated intestinal rings, was not physiologically different in the control and TCDD-treated rats. The time course, optimum concentration and net rate of active transport of amino acid and monosaccharide were similar in both groups of rats. These results suggest that TCDD alters lipid assimilation and, possibly the conversion of lipid to carbohydrate.

Protein Synthesis in Isolated Rat Intestinal Tip and Crypt Cells,

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The intestinal mucosa presents a cell line that is differentiating as it migrates from the crypt to the tip regions of the villus. The relative rates of net protein synthesis in these two cell types are investigted by differential radiolabeling, ^{3}H-leucine–crypt and ^{14}C-leucine–tip, *in vitro*. The radiolabeled proteins of the two cell types are mixed and separated by differential centrifugation into subcellular fractions (SCF), cell debris/nuclei, mitochondria, microsomes and supernate. When normalized to the ^{3}H/^{14}C ratio of the whole cell homogenate (WCH), the microsomes and mitochondria have ratios similar to the WCH, but the ratio of cell debris is higher, and that of the supernate lower. The SCF are then analyzed by SDS-polyacrylamide gel electrophoresis to determine any relationship between molecular weight and relative SCF net protein synthesis in the tip and crypt cells. All SCF exhibit variable rates of net synthesis. The mitochondrial and supernatant fractions exhibit the greatest heterogeneity with respect to ^{3}H/^{14}C (crypt/tip) ratio. This result indicates evolution of mitochondrial and soluble proteins as the crypt cell differentiates to tip. The supernate also exhibits a decreasing molecular weight to decreasing crypt/tip ratio correlation. The phenomenon of decreasing molecular weight correlating with decreasing rate of turnover, observed in liver cytosol *in vivo* pulse-chase experiments, is not a generalized one but varies with cell type and with differentiation.

Effect of Butylated Hydroxyanisole (BHA) on Dose Response for Benzo(a)pyrene Metabolite-DNA Adducts in Mice.

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The phenolic antioxidant, BHA, has been shown to be a potent inhibitor of the neoplastic effects of benzo(a)pyrene (BP) in mouse lung and forestomach. We previously showed that BHA treatment of mice inhibited BP metabolite-DNA adduct formation in the lung to the same degree that BP-induced pulmonary adenoma formation was inhibited.

In this study the formation of BP metabolite-DNA adducts in lung, liver, and forestomach of control and BHA-treated (5 mg/g diet) female A/HeJ mice was examined as a function of BP dose (PO) ranging from 2 to 1350 μmole/kg. The major identified adduct in each tissue at each dose was the 7ß, 8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-tetrahydro-BP (BPDEI)-deoxyguanosine adduct. The 7ß,8α-dihydroxy-9ß,10ß-epoxy-7,8,9,10-tetrahydro-BP (BPDEII)-deoxyguanosine adduct and a BP-phenol-oxide-DNA adduct were also observed. In untreated animals, the dose-response curves for these adducts were sigmoidal in each tissue. In forestomach the dose-response curve for BPDE-DNA adducts levels in BHA-treated mice was parallel to the curve for control animals and, thus,

the inhibition (45%) of adduct formation was independent of BP dose. In contrast, BHA treatment diminished the curvilinear nature of the dose-response curves for BPDE adducts in lung and liver. The dose-response curves in lung and liver of treated animals were approximately linear. The inhibition in lung (70%) and liver (80%) was highest at a BP dose of 0.3 mmole/kg. As BP dose approached zero, the inhibition of BPDE-DNA adduct formation decreased with BP dose and approached values of 40% (lung) and 55% (liver). These results suggest that BHA treatment will also inhibit the neoplastic effects of BP at environmentally relevant doses.

Developmental Correlation between Rat Cytosolic Androgen Binding Protein and Androgen-Inducible Microsomal Monooxygenase Activity. R. C. RUMBAUGH, T. SLOOP, G. W. LUCIER and Z. MCCOY, *Laboratory of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Previous investigations have demonstrated the presence in male rat hepatic cytosol of one or more proteins which bind both androgens and estrogens with high affinity. Furthermore, this protein was shown to be regulated by a complex interaction of gonadal hormones. This hepatic steroid-binding protein may function as an androgen receptor whose ligands control its level with androgen-stimulating and estrogen-repressing receptor synthesis. Many hepatic microsomal, drug- and steroid-metabolizing enzymes are steroid-responsive in a manner analogous to the hepatic androgen-binding protein (ABP). The present study was designed to determine the functional role of hepatic ABP and whether its presence correlates with the presence of androgen-inducible microsomal drug metabolism. Analysis of hepatic ABP was performed using ^{3}H-5-dihydrotestosterone (DHT) as the ligand in intact and hypophysectomized rats of both sexes. Hepatic cytosol from male rats prelabeled with DHT showed a time-dependent translocation of radioactivity to purified hepatic nuclei in a cell-free system. Hepatic ABP of male rats bound to columns of denatured DNA cellulose and was eluted by a salt gradient in three peaks. This DNA binding ability was absent in livers of female rats and reduced in livers of hypophysectomized rats. Hepatic ABP is absent in immature male and mature female rats. In hypophysectomized rats of either sex, the levels of hepatic ABP are greater than those of female rats but less than those of normal males. The presence of hepatic ABP correlated with the ability of androgen to induce hepatic microsomal ethylmorphine *N*-demethylase (EM). EM activity was high in males, low in females, and low in hypophysectomized animals. Treatment of hypophysectomized animals with testosterone did not induce either hepatic ABP or EM demethylase. Treatment of male rats with estradiol feminized levels of both hepatic ABP and EM. The results suggest that the presence of hepatic ABP coincides developmentally with androgen induction of microsomal mixed-function oxidase. The presence of an intact pituitary is required for expression of normal male levels of both hepatic ABP as well as EM demethylase. These observations are consistent with the concept that androgen action in the liver occurs through a receptor mechanism analogous to other steroid-responsive tissues.

Characterization Studies of Renal Brush Border Membrane Vesicle Uptake of Lead. W. W. VICTERY and B. A. FOWLER, *Laboratory of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The mechanism by which lead (^{203}Pb) is transported into renal proximal tubule cells was studied *in vitro* using rat kidney brush border membrane vesicles (BBMV). Incubation of BBMV with carrier lead over a concentration range of 0.05-100 μM did not alter the fractional ^{203}Pb uptake rate, indicating that ^{203}Pb is primarily bound to high capacity sites. BBMV incubated with ^{203}Pb showed greater retention of radioactivity when washed with a buffer containing 1 mM Pb acetate in comparison with 1 mM $LaCl_2$ or 1 mM mannitol. ^{203}Pb efflux studies conducted at various time points following incubation demonstrated that this effect occurred because the 1 mM Pb wash reduced ^{203}Pb release from BBMV. Studies conducted to distinguish between intravesicular uptake and membrane binding of ^{203}Pb showed extensive membrane binding. BBMV preloaded with 200 μMPb acetate also displayed only a 50% reduction in ^{203}Pb uptake indicating uptake across the vesicle membranes against a concentration gradient. In addition, incubation of vesicles at 0°C significantly reduced ^{203}Pb to 50% of the value found at 25°C, thus demonstrating a temperature-sensitive component of the uptake process. These studies indicate extensive ^{203}Pb membrane binding to BBMV but also the presence of an intravesicular uptake mechanism.

Mechanisms Controlling Excretion of Benzo(a)pyrene and Its Metabolites by the Southern Flounder, *Paralichthys lethostigma*. JOHN B. PRITCHARD, MARIAN E. FRECH and JOHN R. BEND, *C. V. Whitney Laboratory, National Institute of Environmental Health Sciences, St. Augustine, FL 32084.*

Marine teleosts extensively metabolize polycyclic aromatic hydrocarbons (PAH) via the cytochrome P-450 mixed-function oxidase system. In contrast to mammals, a much greater fraction, approximately 60%, of the metabolism of the model PAH, benzo(a)pyrene (BP), occurs at the benzo ring. Metabolism in this region of the molecule yields metabolites which have been shown to be toxic, i.e., carcinogenic, mutagenic. Following injection of 2.5 μmole/kg radiolabeled BP, BP-7-phenol, BP-1-phenol, or BP-7,8-*trans*-dihydrodiol, the clearance of each compound exceeded the simultaneously measured glomerula-filtration rate, thus, demonstrated net tubular secretion. Clearance of label following BP-7,8-diol administration exceeded that following the phenol metabolites by 10-fold and was more than 30 times the initial clearance after injection of BP itself. Analysis of the urine by TLC and HPLC indicated that 90% of the urinary radioactivity was found in polar metabolites of the initial compound, predominantly glucuronide and sulfate conjugates. Secretion was blocked by probenecid, the classical inhibitor of the organic anion transport system. Finally, it was shown that other organic anions, including the herbicide 2,4-D, were very effective inhibitors of the secretion of these PAH metabolites, suggesting that these "less toxic" anions might potentiate PAH toxicity by prolonging their retention and promoting bioconcentration.

Proton-Coupled L-Lysine Uptake by Renal Brush Border Membrane Vesicles From Mullet (*Mugil cephalus*). SOON-HO LEE and JOHN B. PRITCHARD, *Whitney Laboratory, National Insti-*

tute of Environmental Health Sciences, St. Augustine, FL 32084.

The uptake of the basic amino acid, L-lysine, was studied in brush border membrane vesicles isolated from mullet kidney. The uptake of lysine was not significantly stimulated by a Na^+ gradient, and no overshoot was observed. However, when a proton gradient (pH_o = 5.5; pH_i = 8.3) was imposed across the membrane in the absence of Na^+, a transient overshoot was produced. When the proton gradient was short circuited by the proton ionophore, carbonylcyanide *p*-fluoromethoxyphenyl hydrazone, proton gradient dependent uptake of lysine was abolished. Kinetics of lysine uptake determined under equilibrium exchange conditions indicated that the V_{max} increased as available protons increased (2.1 nmole/min/mg protein at pH 7.5 to 3.7 nmole/min/mg at pH 5.5), whereas the apparent K_m (4.9 ± 0.6 mM) was not altered appreciably. When membrane potential (inside negative) was manipulated by K^+ diffusion via valinomycin, a similar (but smaller) stimulation of lysine uptake was observed. Furthermore, when the membrane potential and the proton gradient were imposed simultaneously, a much higher overshoot in lysine uptake was shown, and the uptake of lysine was approximately the sum of the components measured separately. These results indicate that the uptake mechanism for basic amino acids is different from that of neutral or acidic amino acids and that the protonmotive force can provide the driving force for the uptake of L-lysine into the isolated brush border membrane vesicles.

Bicarbonate-Gradient-Driven Sulfate Transport by Marine Teleost Renal Tubule Epithelial Membrane Vesicles. J. LARRY RENFRO, WILLIAM A. PARKINSON and JOHN B. PRITCHARD, *C. V. Whitney Marine Research Laboratory, National Institute of Environmental Health Sciences, St. Augustine, FL 32084.*

An inside > outside, 25 mM HCO_3^- gradient stimulates SO_4^{2-} uptake into membrane vesicle preparations predominantly enriched in brush border membranes. At 5 S this gradient produced a five-fold "overshoot" in SO_4^{2-} uptake compared to equilibrium (10 min). The concentrative uptake was unaffected by short-circuiting with 2 μg/mL valinomycin and 100 mM KCl on both sides. It was also uninfluenced by an inside negative electrical potential generated by 100 mM KCl gradient (in > out) in the presence of valinomycin. SO_4^{2-} efflux from these vesicles was stimulated by 25 mM HCO_3^- in the incubation medium. Other anions stimulate SO_4^{2-} uptake in the following sequence: $SO_4^{2-} > HCO_3^- > SCN^- > Cl^-$. HCO_3^- inhibits SO_4^{2-} self-exchange. HCO_3^- stimulation of SO_4^{2-} uptake can be inhibited by Hg^{2+}, 4,4'-diisothiocyano-2,2'-disulfonic acid, stilbene and, partially, by probenicid. From a previous study we have identified a $2H^+$:SO_4^{2-} symport process in basolateral membranes of the telecost kidney. The combined results suggest a tentative model of SO_4^{2-} secretion by renal tubules as follows: peritubular SO_4^{2-} is taken into the cells by $2H^+$:SO_4^{2-} driven by an H^+ electrochemical gradient. SO_4^{2-} exits the cell across the brush border in a neutral exchange with lumenal HCO_3^-. The latter seems to be an anion-exchange mechanism.

Metabolism of Xenobiotics by Clara and Type II Cells Isolated From Rabbit Lung. T. R. DEVEREUX, R. M. PHILPOT, J. D. DILIBERTO, J. R. BEND, B. R. SMITH and J. R. FOUTS, *Laboratory of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Freshly isolated cell fractions containing 80-90% alveolar type II or 50-70% Clara cells were obtained by proteolytic digestion of lung followed by centrifugal elutriation and Percoll density gradient separation of cells. Clara cells metabolized 7-ethoxycoumarin (7-Ec) to umbelliferone at a rate of 180 pmole/mg protein/min, while the 7-Ec deethylation rate in type II cells was 83 pmole/mg/min. Benzo(a)pyrene hydroxylase and epoxide activities were also higher (3-5 times) in Clara cells than in Type II cells. Coumarin hydroxylase activity seemed to be specifically localized in the Clara cell-rich fractions. However, glutathione transferase (benzo(a)pyrene 4,5-oxide as substrate) was about the same in the two cell types. Experiments conducted to examine electron transport in xenobiotic metabolism revealed qualitative as well as quantitative differences between the Clara and Type II cells. Antibodies to cytochrome P-450 reductase and cytochrome P-450 form 2 inhibited 7-Ec deethylation in Clara cell microsomes by only about 50-60%. These experiments indicated that cytochrome b_5 may be involved in the metabolism of xenobiotics in Clara cells to a greater extent than in Type II cells. Also, there may be another (other) cytochrome P-450 isozyme(s) present in Clara cells which has not been identified previously in rabbit lung.

Stereoselectivity and Regioselectivity of Rat Liver Glutathione Transferases with Styrene 7,8-Oxide (SO) as Substrate. C. HARRIS, A. BHATIA, B. YAGEN and J. R. BEND, *Laboratory of Pharmacology,* and O. HERNANDEZ, *Laboratory of Environmental Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Rat hepatic glutathione transferases (GT) were studied for their stereoselectivity and regioselectivity in the enzymatic reaction of glutathione with radiolabeled (R)-SO and (S)-SO. Stereoselectivity was determined for GT forms AA, B, C, D and E. Apparent K_m values for (S)-SO varied over a wide range (4.8-29.1 mM) and appeared to increase with the isoelectric point of the enzyme used while (R)-SO values were much less variable (0.8-2.6 mM) and showed no patterns of increase. K_m values were higher for the (S)-SO enantiomer for each of the enzymes relative to the (R)-SO. Determination of the parameter k_{cat} also showed that the (S)-SO was higher than (R)-SO for each GT tested. At (R, S)-SO concentrations of 0.1 and 1.0 mM, the (R)-SO enantiomer was preferentially converted to the benzylthioether product (1-phenyl-2-hydroxyethyl glutathione) relative to the nonenzymatic reactions. At these same concentrations the (S)-SO enantiomer was preferentially converted to the benzyl alcohol product (2-phenyl-2-hydroxyethyl glutathione). When the substrate concentration was raised to 10 mM SO, the (S)-SO showed a mixed preference for product formation, and the (R)-SO converted to show preference for benzyl alcohol product formation. Stereoselectivity of this class of enzymes for SO, determined by product formation, depended on the GT isozyme used, while the regioselectivity of the reaction is a function of the enantiomer and its concentration. Insights into the catalytic mechanisms for individual GTs, based on established epoxide chemistry, suggest that the rat hepatic GT enzymes are more involved in

catalysis than can be explained by a proximity effect or enhancement of thiol nucleophilicity.

Biochemical Characterization Studies on the 11,500 and 63,000 Dalton ^{203}Pb-Binding Components of Rat Kidney Cytosol.

P. MISTRY and B. A. FOWLER, *Laboratory of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Previous studies from this laboratory have demonstrated the presence of soluble 11,500 and 63,000 dalton ^{203}Pb-binding components in kidney and brain but not lung or liver of control rats. In the present investigations, saturation analysis of the whole cytosol and the two partially purified proteins disclosed an apparent dissociation constant K_d of approximately 3×10^{-8} M for these components. The binding capacity was 43 pmole/mg cytosolic protein and 430 pmole/g kidney. Sucrose density gradient analysis (SDG) of the 63,000 dalton component disclosed a sedimentation coefficient of 4.6 S. Competition studies with other divalent cations on SDG showed a marked (40-80%) displacement by Cd^{2+} and Zn^{2+} and little or no displacement by La^{2+}, Cu^{2+}, Ca^{2+} and Mg^{2+}. Furthermore, the displacement of bound ^{203}Pb by Cd^{2+} and Zn^{2+} was greater with an increase in incubation time. The results of these studies indicate that the previously reported cytosolic Pb-binding proteins of rat kidney are saturable and possess a high affinity for Pb. In addition, the affinity may be altered by the presence of some divalent cations.

Estrogenicity of Zearalenone Mycotoxins in Liver.

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This study investigates binding characteristics of three derivatives of the estrogenic mycotoxin zearalenone by components of liver cytosol. Cytosol from male and female rats contains similar amounts of specific estrogen receptors which sediment in the 8-9 S regions of 5-20% sucrose gradients. In addition, male liver cytosol contains a second class of "Nonreceptor" estrogen-binding sites sedimenting in the 4-5 S region of sucrose gradients. Sedimentation analyses show that each of the mycotoxin derivatives (P-1496, P-1502 and P-1506) behave in an analogous manner to the synthetic estrogen DES, in that they compete effectively for 8 S receptor sites while they bind poorly to 4 S "Nonreceptor" sites.

Relative binding affinities for receptor sites were determined by competition studies. During a short (90 min) incubation period the relative binding affinities of P-1496, P-1502 and P-1506 for receptor sites were 30%, 17% and 12%, respectively, of that exhibited by estradiol-17β. The relative binding affinity of estradiol-17B and P-1496 did not change as a function of time at 4°C. However, the relative binding affinities of P-1502 and P-1560 decreased to 6.8% and 4.2%, respectively, during an extended (18 hr) incubation period at 4°C. Each derivative exhibited similar relative binding affinities towards liver and uterine receptors during the extended incubation period. Dissociation rate constants were obtained indirectly by measuring the rate of exchange of the unlabeled ligand with [^{3}H]-estradiol at 25°C. Treatment of rats with zearalanol (P-1496) resulted in a pronounced increase in hepatic synthesis of circulating triglycerides associated with very low density lipoproteins. Moreover, zearalanol treatment affected protein synthesis in liver in a manner similar to estradiol, as assessed by two-dimensional electrophoresis and autoradiography of proteins newly synthesized by isolated hepatocytes.

These studies show that the mycotoxin derivatives have the potential for modulating liver function through interaction with specific estrogen receptors. The estrogenic potential of the derivatives may depend upon the formation of a stable slowly dissociating ligand-receptor complex.

Epidermal Cell Differentiation and Xenobiotic Metabolism.

R. J. POHL, M. W. COOMES, R. J. SPARKS and J. R. FOUTS, *Laboratory of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Epidermal basal cells differentiate into sebaceous cells and into the cells of the upper epidermis, which elaborate keratin (differentiated keratinocytes) and are eventually exfoliated from the *stratum corneum*. Sebaceous cells are readily identified by their lipid inclusions, and may be separated into two types of different density (heavy and light). Basal (undifferentiated) cells are small in size and have a low protein/DNA ratio due to a paucity of cytoplasm. Differentiated keratinocytes are larger and have a higher protein/DNA ratio. We have found a relationship between stage of differentiation and metabolism of xenobiotics. Heavy sebaceous cells are more active in metabolism of xenobiotics than are light sebaceous cells, and differentiated keratinocytes are more active than undifferentiated basal cells.

Characterization of an Oyster Cadmium-Binding Protein: Structural Studies.

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A soluble, low molecular weight (7,400 daltons) cadmium-binding protein (CdBP) has been isolated from cadmium-treated American oysters *(Crassostrea virginica)* and partially characterized. Unlike metallothionein, the predominant amino acids of CdBP are aspartic (12.9 mole-%) and glutamic (9.7 mole-%). Furthermore, the cysteine content of CdBP is only 7.6 mole-% as compared to 30 mole-% for metallothionein and CdBP contains the aromatic amino acids, phenylalanine (3.5 mole-%), tyrosine (2.6 mole-%) and tryptophan. Although isolated CdBP normally contains only one g-atom of Cd/mole of protein, the addition of excess $CdCl_2$ to the supernatant during isolation maximally increased the amount of bound Cd twofold. At pH 7.35, CdBP exhibits a positive circular dichroic band at 259 nm which is abolished at pH 3.0. This band has been attributed in metallothionein to an extrinsic Cotton effect originating from the Cd-sulfur bond. The circular dichroism of CdBP in the 200-220 nm range indicates that 50% of the protein exists in a β-pleated sheet conformation while the remainder (~40%) is random. These data indicate that although the Cd-S metal-binding sites of this oyster CdBP appear similar to those present in metallothionein, the more ordered structure of CdBP and its lower metal-binding capacity provide further evidence that this protein is not a true metallothionein but represents a distinctly different class of low molecular weight Cd-binding proteins.

Research on Chlordecone (Kepone).

CLIFFORD L. MITCHELL, *Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Over the last two years, the Laboratory of Behavioral and Neurological Toxicology has developed a research program to study the neurobiological substrates for chlordecone neurotoxicity. Eventually, such information should be applicable to the study of other chemically related insecticides and to the development of effective therapeutic strategies for the organochlorine class of insecticides. Research in our laboratory has centered on three main themes: Tremor, neuroendocrine effects, and the neurobehavioral consequences of exposure during development. The most prevalent neurotoxic effect observed in humans following exposure to chlordecone is tremor. We have found that this manifestation can be quantified in a rodent species.

Pharmacological studies suggest that chlordecone may produce tremor by affecting sites in the brain stem and/or spinal cord. Chlordecone and chemically related insecticides have been reported to have effects on endocrine function. Our research suggests that the estrogenic-like activity of chlordecone interferes with the function of the hypothalamo-pituitary system. Chlordecone has a weak affinity for central nervous system estrogen receptors and partially mimics the effects of estrogen in modulating serum levels of LH and prolactin. Chlordecone decreases pituitary enkephalin levels in a manner similar to estrogen. The estrogenic nature of chlordecone may be responsible for alterations in the ontogeny of the pituitary enkephalinergic system when exposure occurs during neuroendocrine differentiation. Chlordecone produces vaginal estrus with a time course similar to that of estrogen. However, chlordecone cannot substitute for estrogen in priming behavioral receptivity and in fact reduces sexual behavior in steroid primed, ovariectomized females.

Long-term functional alterations that may follow exposure to chlordecone during development have not been extensively evaluated. However, data generated in this laboratory have demonstrated that exposure to chlordecone during development affects several neurological functions having survival value for the organism. Reactivity and emotionality characterized by changes in startle responsiveness and serum steroid levels have been observed in chlordecone-exposed animals. Such changes may be responsible for subtle alterations in the ability of chlordecone-exposed animals to perform certain types of learned tasks. In addition, our research has demonstrated that neonatal exposure to chlordecone results in subtle long-term alterations in neurological functioning. Adult animals exposed as neonates to chlordecone may appear relatively normal under some types of testing conditions, but underlying neurological deficits can be unmasked by various environmental conditions or stressors. Future studies will attempt to (1) determine the site(s) and mechanism(s) for chlordecone-induced tremor, (2) characterize in greater detail its effects on hypothalamo-pituitary function, and (3) examine the possible mechanism(s) by which chlordecone affects the development of neurobehavioral functioning.

Quantification and Mechanism of Action of Chlordecone-Induced Tremor in Rats.

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In humans, tremor is a frequent sign of exposure to insecticides and other environmental toxicants. Our laboratory has developed an animal model to study tremor using a prototypic organochlorine insecticide, chlordecone (Kepone). Male Fischer-344 rates were given chlordecone by intraperitoneal injection and tremor measured by a noninvasive technique utilizing a spectral analyzer. Tremor produced by chlordecone was found to be dose- and time-related and had a peak frequency of approximately 11.9 cycles/sec. The tremor produced by chlordecone could be differentiated from that produced by pharmacological tremorgens (harmine and oxotremorine) or an agent that produces stereotypic behavior (apomorphine). Pharmacological studies were then initiated to study the possible neurobiological substrate underlying chlordecone-induced tremor. Extrapyramidal systems (caudate and cerebellum) that control motor movement and posture did not appear to be necessary for the expression of chlordecone-induced tremor, while spinal and supraspinal pathways are required. Exposure to chlordecone appears to activate higher CNS processes which may contribute to the manifestation of tremor. Serotonergic, gabaminergic, and cholinergic, but not catecholaminergic, systems appear to contribute to chlordecone-induced tremor. Studies are now underway to define more precisely the role that higher CNS processes may play in the control of this neurological manifestation.

Estrogen-like Activity of Chlordecone on the Pituitary Enkephalin System of Rats.

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It has been clearly demonstrated that chlordecone exerts estrogen-like effects on the reproductive system. However, the extent to which the estrogenic activity of this neurotoxicant may contribute to its central nervous system effects are not clear. The purpose of this study was to compare the estrogenic activity of chlordecone and estrogen on the rat hypothalamo-pituitary axis (HPA) by using the pituitary enkephalin system as a model. It was found that the enkephalin system of the anterior pituitary is regulated by circulating estrogen. Ovariectomy caused an increase in the pituitary content of enkephalin and this increase was prevented by estrogen treatment. Administration of estrogen reduced the pituitary content of this peptide in intact male rats. Similarly, chlordecone treatment reduced the pituitary content of enkephalin in a manner identical to that of estrogen. A single injection of chlordecone (75 mg/kg, IP) caused a delayed decrease in the enkephalin level of the anterior lobe (but not the posterior lobe) of the pituitary. This effect of chlordecone was selective to male rats since the pituitary enkephalin level of females was not altered by such treatment. Levels of other pituitary hormones, such as β-endorphin or vasopressin, were not decreased. Further studies revealed that the similarities between chlordecone and estrogen could be extended to other pituitary hormones. For example, implantation of estrogen or of chlordecone to ovariectomized rats caused similar changes in serum prolactin and luteinizing hormone levels. Thus, this study demonstrates a clear estrogenlike action of chlordecone on the HPA. Moreover, due to the similarity of both estrogen and chlordecone to antidopaminergic agents in altering [met^5]-enkephalin levels in both the pituitary and caudate

nucleus, it is speculated that a dopaminergic mechanism may be responsible for their actions on enkephalin system.

Neurobehavioral Consequences of Developmental Exposure to Chlordecone. C. F. MACTUTUS, H. A. TILSON, and K. L. UNGER, *Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The developing organism is typically regarded as potentially more susceptible to xenobiotic compounds than the mature adult. The relative persistence of chlordecone in the environment and its detection in human milk suggests a continuing source of exposure for the developing human. The present study investigated the potential risk of developmental exposure to this organochlorine compound for appropriate maturation of behavioral and neural function. We have shown that neonatal exposure to chlordecone produces a toxicological syndrome in preweaning rats similar to that observed in exposed humans. Analogous measures in adulthood indicated little evidence for any residual effects, with the exception of a sex-dependent alteration in body weight. However, the use of pharmacological manipulations revealed that long-term alterations were indeed present in adulthood. Collectively, these results indicate the persistence through adulthood of alterations in each component of the preweaning toxicological syndrome.

DNase I Hypersensitive Sites and S1 Nuclease-Sensitive Sites in and around the Seminal Vesicle Secretion Gene IV in Nuclei from Castrate Rats and Rats Treated with Testosterone. S. HARRIS and C. TENG, *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The rodent seminal vesicle, one of the male sex accessory glands, is dependent upon androgens for maintenance of its structure and function. The secretory proteins and fluid of the seminal vesicle make up the bulk of semen. We have been working the past several years on two of these testosterone-dependent secretory proteins referred to as seminal vesicle secretion IV and V (SVS IV and SVS V). The genes for these proteins have been isolated from a λ rat gene library by using radioactive probe derived from their respective messenger RNAs. The entire SVS IV gene (or more properly, "transcription unit") and some 5′-flanking and some 3′-flanking DNA have been completely sequenced. A region about 110 base pairs (bp) upstream (5′) from the initiation site for transcription (CAP site) can potentially form a palindromic structure (inverted repeat) consisting of a 9 bp stem and a 7 base loop. It is DNA and RNA structures similar to these inverted repeats which have been shown to have high potential as specific binding sites for various regulatory macromolecules. The position of this palindromic structure (− 110 bp from the CAP site) in the SVS IV gene has been shown in other eukaryotic gene system (− 50 to − 500 bp) to be of some importance in proper gene expression. A plasmid, referred to as pSVS 3.3, contains a 3.3 kb Eco RI fragment cloned into the Eco RI site of pBR325. On that 3.3 Kb Eco RI fragment are the entire transcription unit (1950 bp) for SVS IV precursor mRNA, about 150 bp of 5′-flanking, and 1200 bp of 3′-flanking, DNA. In the supercoiled plasmid of pSVS 3.3 conditions under which the DNA is under a state of B → Z configurational change (stressed), the palindrome at ~ − 110 bp upstream seems to form, as assayed by the loop becoming S1 nuclease sensitive. We also present data on sites in chromatin structure which are S1 nuclease-sensitive and DNase I-hypersensitive. These sites seem to vary whether the animal has seen testosterone or has been castrate for several weeks. The suggestion is various chromosomal proteins may specifically interact with structures similar to the palindrome discussed above and alter the transcriptional properties of these genes whose expression is dependent upon androgens.

Expression of V-ras Oncogene in a Primary Human Prostate Cell Line LnCap. D. CARTER, J. HOROSZWEICZ, and D. MICKEY, *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The risk of prostate cancer in man increases exponentially after age 60. Thus far the basic mechanism involved in the genesis of prostate cancer has not been elucidated. Recently it has been shown that human bladder carcinoma DNA transfers a gene homologous to the transforming gene of the Harvey murine sarcoma virus oncogene, v-ras, in transfection experiments.

We have investigated the expression of this gene or of a gene homologous to the v-ras gene in prostate carcinoma cell lines which were derived from primary and metastatic tumors. In addition we have investigated protein synthetic patterns in the cell lines. The protein spectra observed do not show the synthesis of major differentiated products. One of the cell lines is responsive to androgens however. The RNA from the androgen responsive cell line, LnCaP (primary tumor cell line) appears to contain hybridizing sequences for the v-ras probe. The RNA from benign hyperplastic tissue or the metastatic cell lines appeared to contain non-detectable levels of the v-ras sequences. It is apparent from the protein synthetic patterns that the cell lines differ in the spectra of gene products expressed.

The detected expression of the v-ras gene in the LnCaP cell line may indicate a mechanism of transformation in common with the other urogenital cell lines already known to express this gene.

Allelic Variation in the SVS IV Gene Resulting from an Intronic Insertion. B. DICKSON and S. HARRIS, *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The seminal vesicle secretion IV (SVS IV) gene in rats shows two variant patterns in restriction enzyme digests of genomic DNA. The variants differ by the presence of a 200 base-pair insertion in certain restriction fragments, although no variation has been detected in the cDNA or the secretory protein. Phage clones for two forms of the gene have been recovered from a rat genomic library. Restriction maps of the various clones compared to the cDNA have localized the insertion to the second intron of the gene. The DNA sequence of the insertion is composed of 8-10 direct repeats of 20 base pairs. Two phage clones for the SVS IV gene were recovered from a single, plaque purified, hybridization signal using ^{32}P-cDNA SVS IV as a probe. The clones differ only in the

presence of the 200 base-pair insertion. Otherwise identical restriction maps and DNA sequence data indicate the shorter clone was derived from the longer clone by excision of the 200 base-pair fragment. Ten inbred rat strains and a random bred population have been examined for the presence of these DNA variants. All the inbred strains were fixed for the variant containing the insertion. Heterozygous rats from the random bred population were crossed and the progeny showed segregation of the alleles in single gene Mendelian fashion. The repeated sequence insertion thus causes a stable allelic variant in the SVS IV gene.

Use of Human Embryonic Palatal Mesenchyme Cells (HEPM) to Study the Effects of Environmental Chemicals on Cell Proliferation. W. WILLIS, R. GROVE, and R. PRATT, *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Testing for potential teratogenicity using the FDA-segment II protocol is expensive, time consuming and capable of handling only a small percentage of the environmental chemicals which need to be investigated. A rapid, sensitive and inexpensive means to prescreen potential teratogens, such as using embryonic cells in culture, is needed. The development of the mammalian secondary palate offers a number of developmental events which could be used in such an assay. We have established a human embryonic palatal mesenchyme (HEPM) cell line and find that these cells represent the undifferentiated fibroblast-like cells from the palatal shelves. Their growth in culture is rapid and occurs well with added growth factors in the absence of serum. Potential teratogens are introduced at various concentrations one day after initial plating and the final cell number is determined after 3 days of growth. The HEPM cells are extremely sensitive to teratogens which inhibit DNA synthesis or mitosis which results in reduced cell number. Teratogens such as glucocorticoids, colchicine, or 5-fluorouracil inhibit growth in a dose-dependent manner. HEPM cells can be generated in large numbers at a minimal cost, and the endpoint (cell number) can be easily determined. These cells display unique sensitivities of a number of human tissues or organs which are commonly malformed including the lip, palate, limb and heart. If the HEPM cells are used in conjunction with Braun's tumor cell attachment assay for teratogens in order to detect those teratogens not screened by the tumor cell assay, this system may fulfill the requirements of a rapid, inexpensive screening assay for potential environmental teratogens.

Growth and Differentiation of Secondary Palatal Epithelial Cells in Culture. R. I. GROVE and R. M. PRATT, *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The role of the epithelium in the development of the secondary palate is crucial, since it functions in the recognition, adhesion, and programmed cell death steps which result in the fusion of the apposing palatal shelves. Certain hormones and growth factors have been proposed to play a role in secondary palate development. In order to more easily investigate the morphological and biochemical effects of these hormones and growth factors on the palatal epithelium, we have developed conditions which permit growth and differentiation of mouse secondary palatal epithelial cells *in vitro*. In the presence of Ham's F-12: Dulbecco's modified Eagles medium (1:1) plus 10% fetal bovine serum and epidermal growth factor (EGF, 20 ng/mL), dissociated day 13 mouse palatal epithelia adhere to plastic culture dishes and begin to spread within 24 hr. By 3-4 days, the cells differentiate into ciliated nasal epithelium and stratified, keratinized oral epithelium. The medial-edge epithelial cells appear to undergo terminal differentiation and cell death by day 4 of culture. Epithelia were also cultured in the presence of serum-free, hormone-supplemented medium which consists of Ham's F-12 plus insulin (10 μg/mL), transferrin (10 μg/mL), selenium (10 ng/mL), and EGF (20 ng/mL). In this defined medium, the epithelial cells differentiate normally but growth is substantially reduced compared to epithelial cells cultured in serum containing medium. Our results have shown for the first time that growth and differentiation of the palatal epithelium can be studied in culture in the absence of the mesenchyme. EGF appears to be essential for palatal epithelial cell growth and differentiation in culture.

Diethystilbestrol Metabolites and Analogs: Biochemical Probes for the Study of Differential Uterine Estrogen Responses. K. S. KORACH and C. FOX-DAVIES, *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Estrogen stimulation of the uterus elicits a spectrum of biochemical responses which are customarily linked together. DES and certain structural analogs, indenestrol A (IA), indenestrol B (IB), indanestrol (I), and pseudo DES (PD) were used as probes to investigate various hormonal responses previously considered interrelated, most notably the events of DNA synthesis and mitosis. These compounds have been shown to have poor uterotrophic activity, except for I, they interact specifically with mouse uterine estrogen receptors (ER) with high affinity. All translocate stoichometrically similar amounts of ER complex to the nucleus. During ER replenishment IA and IB cause recycling but no ER synthesis. PD showed a delayed recycling and synthesis some 10-12 hr after DES. Induction of mouse uterine G-6-PD by these compounds gave responses ranging from 80-185% control with the following pattern of relationship: $I < PD < IA \leq IB \leq DES$. Induction of cytosolic progesterone receptor (PR) was quantified after stimulation with the analogs and gave a range of stimulation of 2-12 pmole/mg protein with the indenestrol compounds being most effective and showing this rank order: $I < PD < DES < IA \leq IB$. Stimulation of DNA synthesis by these compounds ranged from 100-150% control with the following rank order: $I << PD \leq IA < IB << DES$. Thymidine autoradiography indicated nuclear labeling was occuring primarily in luminal epithelium (LE). Uterine mitotic index was determined for the analogs using colchicine arrest. Hyperplastic effects were seen in LE with none in the stroma. The mitotic index ranged from 2/section for PD to 42/section for DES with the following order: $PD << I << IB < IA < DES$. Use of PD has indicated the stimulation of mitosis and DNA synthesis are not necessarily correlated as previously thought for hormone responses and may require two separate receptor interactions. Such a probe should be useful in studying the individual events involved in estrogen induced uterine growth. Secondly, these data indicate that induction of protein synthetic events as evidenced by ER, PR, and G-6-PD are not

coupled as observed by the differential stimulation. Therefore, stimulation of certain uterine responses may depend on the particular ligand receptor complex formed and its interaction may be regulated by specificity at the genomic acceptor site.

Potentiation of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-Induced Prostatic Aryl Hydrocarbon Hydroxylase Activity by DNA Modifiers. I. P. LEE, *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

Enzyme induction is one mechanism by which organisms respond to exposure to drugs and other environmental chemicals. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is known to alter gene expression in various tissues and is a useful model to study underlying biological mechanisms. We have previously reported that aryl hydrocarbon hydroxylase (AHH) in rats can be induced 200-fold by a single dose of TCDD. This paper reports the effects of DNA-modifying agents on the induction of prostatic AHH by TCDD. Male CD rats were pretreated with a single IP dose of either procarbazine (100 mg/kg), busulfan (43.5 mg/kg), methyl methanesulfonate (90 mg/kg) or triethylenemelamine (1.5 mg/kg) prior to a single oral dose of TCDD (10 μg/kg). Pretreatment with any of these agents potentiated the normally observed TCDD-induced AHH activity by approximately 5-fold (approximately 500 to 1,000-fold that of controls). When pretreatment with any of these agents was not followed by TCDD administration, AHH activity was either inhibited or not significantly altered from that of controls.

In order to elucidate possible mechanisms of actions, transcriptional activity of AH-structural gene was studied following treatment of animals with either TCDD or TCDD-plus DNA modifying agents. Cytochrome P_1-450 mRNA was measured by hybridization to a pAhP-2.9[^{32}P]DNA probe which was derived from EcoR I digestion of pAhP-1, a 15.5K BP fragment of mouse cytochrome P_1-450 structural gene. This 2.9K BP fragment represents 5′ region of the P_1-450 gene, subcloned in pBR322. In prostate glands, 23S and 21S mRNA were found to hybridize with a pAhP-2.9[^{32}P]DNA probe. Prostatic P_1-450 mRNA levels 24 hr after either TCDD alone or TCDD-plus procarbazine were 18 and 30 times that of control, respectively, and thus reflected well the magnitude of AHH induction. 23 mRNA in control, TCDD and TCDD-plus procarbazine treated animals were 2-fold greater than that of 21S mRNA. Because of extensive homology between the 21S mRNA and pAhP-2.9, as well as differential induction of the 21S mRNA and P_1-450 mRNA by TCDD or TCDD-plus procarbazine, it is suggested that the 21S mRNA encodes an inducible P-450 protein other than P_1-450. Further studies are needed to fully understand the mechanisms of potentiation of TCDD-induction of AHH activity by DNA modifying agents.

Normal and Abnormal Development of the Rodent Embryo in Culture. E. H. GOULDING, L. D. MCNEW, E. L. PERRY, C. S. KIM and R. M. PRATT, *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

The culture of postimplantation rodent embryos *in vitro* offers several unique advantages in that it eliminates the complex interactions among the maternal-placental-embryonic tissues and teratogenic agents can be tested in order to ascertain their ability to directly interfere with embryonic development. Techniques have now been refined so that growth and development of early cultured embryos compares quite favorably to growth *in vivo*. Presomite rat embryos (day 9.5) or early (3-5) somite mouse embryos have been used for most of our studies on *in vitro* teratogenicity. Embryos were cultured for 48 hr in rat serum and then examined by a recently developed scoring system consisting of 17 morphological features which provide a quantitative estimation of embryonic growth and development. The steroidal alkaloid Jervine, found in various plants, produced a dose-responsive increase in forebrain malformations in the absence of overall growth retardation. Embryos exposed to the anticonvulsant valproic acid *in vitro* exhibited a statistically significant decrease in all growth parameters as well as a high percentage of dysmorphogenesis of the neural tube (exencephaly). The synthetic glucocorticoid triamcinolone, a potent cleft palate inducer *in vivo*, produced a high percentage of cleft lip when exposed to day 10 mouse embryos for 48 hr in culture. In contrast, a high percentage of cardiac malformations were observed when day 8 embryos were treated for 48 hr in culture. Whole embryo culture offers an exciting approach in which to investigate numerous parameters of normal as well as abnormal embryonic development under highly controlled conditions.

Differences in Peroxidase-Mediated Metabolism Among Structural Analogs of Diethylstilbestrol (DES). G. H. DEGEN and J. A. MCLACHLAN, *Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.*

DES has been shown to induce neoplastic transformation in Syrian hamster embryo fibroblasts in culture. DES is metabolized by these cells. The metabolic profile is consistent with peroxidase mediated oxidation found in estrogen target tissue. Structural analogs of DES have varying transforming capacities in this cell system. To evaluate the role of metabolism in the carcinogenicity of DES, the peroxidase mediated metabolism of these analogs was studied. Thus, appropriate compounds (0.01 mM/0.1 mM) were incubated for 1 hr with 300 mU horseradish-peroxidase and H_2O_2 (0.02 mM/0.33 mM) at pH 7.2. Extracts of incubations were analyzed by HPLC and, in some cases, HPLC/MS. Under these conditions DES, tetrafluoro-DES, Z, Z-dienestrol and dimethylstilbestrol were enzymatically oxidized (80, 87, 39, and 72% of parent compound converted). On the other hand, hexestrol, dimethoxy-DES, and E,E-dienestrol were not appreciably oxidized under the same incubation conditions (9, 5, 5%, respectively). These experiments clearly demonstrate differences in the metabolism among these DES analogs. It is noteworthy that those compounds which are extensively oxidized by peroxidase *in vitro* also transform Syrian hamster embryo fibroblasts, while those for which peroxidase-mediated oxidation could not be demonstrated, do not.

Susceptibility of Testicular Tissues to Early Postnatal Treatment With Antineoplastic Agents. R. DIXON, R. BECHTER, M. MATTER, H. WEBER and R. ETTLIN, *Laboratory of Reproductive*

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Unique susceptibility to chemical toxicity is critical to defining hazards and analyzing risks. Testicular development, because it involves both pre- and post-natal periods and includes the differentiation of various tissues, offers a number of potential targets for chemicals capable of perturbing biological processes. Thus, we undertook to determine whether anticancer agents, selected for their mechanisms of action, would produce differential toxic effects on spermatogenic (Sp), Sertoli (S) and Leydig cells (L) if administered acutely on selected postnatal days. Male Sprague-Dawley rats were treated IP once with doses of anticancer drugs (cyclophosphamide-C, cytosine arabinoside-CA, vincristine-V, procarbazine-P or doxorubicine-D) on postnatal day 6, 16, 24 or 45. Preliminary data indicate that: V (all four treatment days) and C (day 16, 24, 45) delay puberty; C (16, 45); CA (16, 24, 45) and V (6) increase reabsorptions; V (45) reduces sperm counts; C (16) and V (16, 24, 45) cause sterility in some of the animals. Reduced epididymal weight is found with C (16, 24) and V (24, 45). Histologic evaluations suggest an association between damage to a particular developing cell type and an observed dysfunction.

Differential Regulation of Protein Synthesis by Estrogens in Component Tissues of the Reproductive Tract.

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The rodent uterus contains three main tissue compartments: Epithelium, stroma, and myometrium. These tissues differ qualitatively and quantitatively in their responses to estrogen stimulation. Studies using the entire mouse uterus have shown that estrogens induce *de novo* protein synthesis in this target organ. In order to determine the influence of estrogen on the different uterine tissues the following procedure was used: Control and estrogen-stimulated uteri were incubated with ^{35}S-methionine for 2 hr.; the three tissues were separated using nonenzymic procedures; proteins from the tissues and the incubation medium were analyzed individually by one and two-dimensional gel electrophoresis; labeled proteins were visualized by fluorography and examined in two ways. Firstly, to determine hormonal influences on individual tissues, a comparison was made between protein patterns from control and estrogen treated preparations. Estrogen treatment enhanced synthesis of three proteins within the stroma (Molecular weight in kdaltons/isoelectric point) (30/5.5; 32/5.5; 33/5.5), one epithelial protein (54/5.2), and a series of proteins in the incubation media (79/6.1; 79/6.2; 79/6.3; 62/5.8). Estrogen treatment did not enhance specific protein synthesis in the myometrial fraction. Synthesis of several proteins within the epithelial, stromal, and myometrial preparations appeared to be depressed by estrogen treatment. Secondly, protein patterns from the various tissues were compared. This showed that the above mentioned proteins were predominantly found in certain tissue compartments. Previous studies of estrogen-induced protein synthesis in whole mouse uterus have yielded conflicting results. However, using a tissue separation procedure we have shown that estrogen sensitive proteins are present. Furthermore, it appears that estrogens stimulate the synthesis of different intracellular proteins in each compartment. Several labeled proteins were found in the media, indicating that the uterus may also respond to estrogen stimulation by rapidly synthesizing and secreting certain proteins. Studies of the interrelationships between these intracellular and secreted proteins should enhance our understanding of estrogen action in the component tissues of the uterus.

Cell Culture Model for the Study of Diethylstilbestrol (DES) Carcinogenicity.

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A normal diploid mammalian cell culture system was used as a model to study the carcinogenicity, mutagenicity, estrogenicity, and metabolism of DES. Syrian hamster embryo (SHE) fibroblasts treated with 0.1, 1.0 and 10 μg/mL of DES gave rise to morphologically transformed colonies at a frequency up to 0.3% of the surviving colonies. The transformed clones were isolated after 20 passages, injected into weanling hamsters, and became tumorigenic after a latency period of 6 to 16 weeks. When SHE cells were treated with benzo(a)pyrene (BaP) at 1.0 and 10 μg/mL, colonies that were morphologically transformed at a frequency up to 0.9%, were isolated and subsequently became tumorigenic. Although the DES and the BaP lines were morphologically indistinguishable in the transformation assay, a concomitant study of somatic mutation at the hypoxanthine guanine phosphoribosyl transferase and the Na^+/K^+ ATPase loci resulted in no induction of gene mutation with DES-treated cells, whereas induction was seen with BaP-treated cells. This *in vitro* transformation assay was used to evaluate other DES analogs and natural estrogens to elucidate the active structural metabolite which causes neoplastic transformation. Tetrafluoro-DES, Z,Z-dienestrol and estradiol catechol transformed these cells, but hexestrol, dimethoxy DES, dimethyl-DES, and E,E-dienestrol did not. Under the transformation culture conditions, cell proliferation was not enhanced by DES. In fact, DES was cytotoxic at 10 μg/mL. Growth curves using submaximal concentrations of fetal calf sera also indicate that DES does not enhance SHE cell proliferation. Thus, these results suggest that, in this model system, DES and related compounds are carcinogenic without any enhanced growth promotion.

Exposure of Diethylstilbestrol during Pregnancy Permanently Alters the Ovary and Oviduct.

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To determine the effects of transplacental exposure to diethylstilbestrol (DES) on the ovary and oviduct of the CD-1 mouse, timed pregnant mice were injected subcutaneously with DES (100 μg/kg) on days 9 through 16 of gestation and female offspring sacrificed from 4 weeks to 10 months of age. Following DES exposure, ovarian alterations such as inflammation, a prominant interstitial compartment composed of medullary tubulelike structures, intra- and para-ovarian cysts from mesonephric remnants, and changes in steroidogenesis were observed. In addition, there were oviductal abnormalities including malformation. As reported previously, the oviduct was closely adherent and coiled around the ovary

in a similar position to that seen in the fetal mouse. This malformation was termed developmental arrest of the oviduct (DAO) and was a consistent finding in female offspring exposed prenatally to DES (100 μg/kg). Increased prevalence of salpingitis and microscopic alterations in the oviduct were also observed. Oviductal epithelium was mostly secretory type with basal vacuoles. In some cases, oviductal epithelium was hyperplastic and formed mucosal folds resembling glands which extended through the muscularis (diverticulosis). The extent of the adenomatous mucosal folds and the degree of extension through the muscularis increased with the age of the animal (100% at 10 mos.). Some characteristics of this abnormality resembled salpingitis isthmica nodosa, a lesion described in women which is associated with ectopic pregnancies and subfertility. Gross and microscopic changes in the oviduct were more consistent than were the changes among other portions of the reproductive tract of DES-treated mice previously reported. Since subfertility has been described in this mouse model as well as in prenatally DES-exposed women, the data presented in this report may help in evaluation of the reported reduced fertility in exposed patients as well as other infertility patients.